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RESEARCH ARTICLE

RP-HPLC Method Development and Validation for Estimation of Solifenacin Succinate in Pharmaceutical Dosage Forms

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ABSTRACT

A new, accurate, precise, and robust RP-HPLC method was developed and validated for the determination of solifenacine succinate in tablet dosage form. The chromatographic separation was achieved on an Water symmetry shield RP_8, (250 m m x 4.6 mm, 5µm or equivalent) stationary phase maintained at ambient temperature. With a mobile phase A: Mix Buffer solution and Acetonitrile in the ratio (70:30) filter through 0.45 µm membrane filter and degase. Mobile Phase B : Mix Buffer solution and Acetonitrile and methanol in the ratio (50:50). Buffer solution: Dissolve 3.4 g potassium dihydrogen phosphate into 1000ml of water. Then add 1ml of Triethylamine, Adjust the pH to 3.0± 0.05 by phosphoric acid. At a flow rate of 1.0 ml/min, and the detection was carried out by using UV detector at 220 nm. Linearity was observed in concentration range of 50.200-150.600µg/ml .Injection volume: 10µl, Run time: 8.45 min. The percentage purity was found to be 98.30.The validation parameters specificity, Accuracy, Linearity, LOD, LOQ and Robustness were studied. With good Correlation coefficient Value of 0.9996.The method was Accurate, Precise, Robust and rapid. The performance of the method was validated according to the present ICH guidelines.

Keywords: RP-HPLC, Solifenacine Succinate, Validation and Method Development

INTRODUCTION

Solifenacin succinate is a competitive muscarinic acetyl choline receptor antagonist used in the treatment of overactive bladder with or without usage incontinence. Chemically it is 1-azabicyclo {2.2.2 } oct-8-yl (1S)-1-phenyl-

3,4-dihydro-1H-isoquinoline-2 carboxylate. Shown in Figure 1. The molecular formula of solifenacin succinate is C 23 H 26 N2 O 2 with its molecular weight 364.46. Solifenacin is extensively metabolized in the liver. The metabolites observed as one pharmacologically active metabolite (4 R- hydroxy solifenacin), and three pharmacologically inactive metabolites (N- glucuronide and the N-oxide and 4 R-hydroxy solifenacin) occurring at low concentrations in human plasma after oral dosing. After oral administration of vesicare to health volunteers, peak plasma levels (Cmax) of solifenacin are reached within 3 to 8 hours after administration and at steady state ranged from 32.3 to 62.9 mg/mL for the 10 mg vesicare tablets ,respectively. The terminal elimination half –life of SF is approximately 45-68 hours. Solifenacin is approximately 98% (*in vivo*) bound to human plasma proteins, principally to alpha -1-acid glycoprotein (1- 10). Literature survey reveals that quantification of solifenacin in human plasma (11,12) rat plasma (13) pharmaceutical compounds (14 -17) ,and industrial waste streams (18) was reported. These methods were reported by using LC-MS/MS (11,12,18) , HPLC (13-16) and HPTLC (17) ,Among all, quantification of solifenacin by LC-MS/MS in biological matrices (11-13) was proved best result. The reported HPLC methods (13-16) have some draw backs in term of ruggedness, reproducibility, and sensitivity in long run. The main goal of the present study is to develop and validate the noval simple, higher sentive, selective, rugged, and reproducible analytical method for quantitative determination of solifenacin in pharmaceutical compounds by HPLC. The developed method would be applied in finished product and in quality control (19).
C 23 H 26 N2 O 2

MATERIALS AND METHOD

System Suitability: System suitability was performed repeatedly inject 10µl of standard solution of solifenacin succinate six times and record the chromatograms and peak responses as directed for procedure for the peak due to solifenacin succinate. The RSD of peak areas of solifenacin succinate for standard replicates is NMT 2.0%.

Specificity: Specificity of method was performed by analyzing a placebo solution, diluent, standard and test solution of Solifenacine succinate tablets.

Identification: The major peak of solifenacin in the chromatogram of the test solution has a retention time, which corresponds to those of the respective major peaks in the chromatogram of standard solution.

Placebo interference: Diluent, placebo solution and sample solution were injected and recorded the chromatodram. No any peak was observed at the retention time of solifenacin and No interference due to the placebo, indicating that method is specific for the assay of solifenacin in vesitone tablets.

Calibration Curve (Linearity): Linearity test result gives assurance that method is valid for its intended use throughout the specified ranges. Incremental concentration of standard solution for active substance was prepared in the working range i.e. about 50% to 150% of the analyzing concentration (target concentration) to verify that the response is proportionately linear. Solutions of solifenacin succinate standard of different strength were prepared and chromatograph; corresponding peak areas were recorded and the calibration graph was drawn for solifenacin succinate by plotting response (peak area) against concentration. The final concentration in the range of (50.200 µg/ml to 150.600 µg/ml in which method were linear.

Precision

Repeatability of (Day 1): The precision of the assay method was determined by analyzing 6 test samples prepared as directed in the test method. The %RSD of 6 results of assay should not be more than 2.0%.The % of relative standard deviation of six test results are within the acceptance limit, indicating that the good precision of the test method.
Intermediate precision (Day 2): The precision of the assay method was determined by analyzing 6 test samples prepared as directed in the test method in another lab. The % RSD of 6 results of assay should not be more than

3.0%.The % of relative standard deviation of six test results are within the acceptance limit, indicating that the good reproducibility of the test method.

Accuracy or (Recovery): The accuracy or recovery of the method was determined by adding standard solution of solifenacin to an aliquot of placebo solution prepared with placebo of Solifenacine succinate tablets (10mg strengths).This solution was then treated as test solution and the amount of solifenacin was determined against a standard solution. The results of % recovery of nine samples were shown that a good recovery of solifenacin from the samples matrix. True value is embraced by the 95% confidence interval, thus, the obtained results of recovery close to true value and the method is accurate for the assay of solifenacin succinate tablets. The accuracy were carried out by 60%, 100%, 140% to determine the recovery study.

Robustness: Robustness of this method was determined by deliberately changing the method parameters, like flow rate, column temperature etc. The results shown that slightly changes of the chromatographic condition do not effect on the assay results and no interference was observed. Thus the chromatographic condition is suitable for the assay and identification of solifenacin succinate tablets. Robustness study was done by changing the Column temperature (25ºC-35ºC), flow rate (0.8-1.2 ml/min) in the drug. % RSD was within the limit as per ICH guideline.

LOD and LOQ: Limit of detection was carried out in Solifenacin succinate the value are found to be 3.14 respectively. Limit of quantification was carried out in Solifenacin succinate the value are found to be 14.16 respectively.

CONCLUSION

In the present study, an attempt was made to develop a simple, accurate, selective and sensitive RP-HPLC method of Solifenacine succinate in pharmaceutical analysis. This method is the only reported method up to date for the determination of Solifenacine succinate in pharmaceutical dosage forms. The method was validated for selectivity, accuracy, linearity, precision (Repetability of (Day.1), Intermediate precision (Day.2) sensitivity, robustness and ruggedness in accordance with ICH guidelines. The results from stress testing, including Separation of the degradation product and quantification of Solifenacine succinate after exposure to stress conditions show the method is stability-indicating and capable of determining Solifenacine succinate in presence of its degradation products. This indicates the selectivity of the method. A simple mobile phase without preparation of any buffer solution or adding ion-pairing agents and a short run times are advantageous and make this method suitable for routine analysis of large number of samples per day.

Acceptance Criteria

1.For LOD S/N (signal to noise ratio) should be ≥3 &≤9
2.For LOQ S/N (signal to noise ratio) should be≥10 &≤30

REFERENCES

1. F.J.Morales –Olivas and L.Estan, Solifenacin pharmacology Archivos Espanoles de Urologia, vol.63,no 1,pp.43-52,2010.
2. O.Doroshyenko and U. Fuhr, Clinical pharmacokinetics and pharmacodynamics of solifenacin, Clinical Pharmacokinetics, vol.48,no 5,pp.281-302,2009.
3. S.Hoffstetter and C.L. Fah, Solifenacin succinate for the treatment of overactive bladder , expert opinion on Drugs Metabolism and Toxicology , vol .5 ,no.3,pp.345-350,2009.
4. M.E. Kuipers, W.J.J. Krauwinkel, H . Mulder, and N Visser, Solifenacin demonstrate high absolute bioavailability in health men, Drugs in R and D , vol.5, no.2,pp.73-81,2004.

5. U.L. Roberti Maggiore, S. Salvatore, F. Alessandri et al.,'' Pharmacokinetics and toxicity of antimuscarinic drugs for overactive bladder treatment in females, '' Expert Opinion on Drug Metabolism and Toxicology, vol. 8, no.11, pp. 1387-1408, 2012.
6. T. Uchida, W. J.Krauwinkel, H. Mulder, and R.A. Smulders, '' Food does not affect the pharmacokinetic of solifenacin, a new muscarinic receptor antagonist; results of a randomized crossover trial,'' British Journal of Clinical Pharmacology, vol.58, no.1,pp.4-7,2004.
7. S .Yamada, S. Kuraoka, A. Osano, and Y. Ito, '' Characterization of bladder selective of antimuscarinic agents on the basis of in vivo drugs-receptor binding,'' International Neurourology Journal, vol. 16,no.3,pp.107 - 115,2012.
8. S. Maruyama, H. Tsukada, S. Nishiyama et al ., '' In vivo quantitative autoradiographic analysis of brain muscarinic receptor occupancy by antimuscarinic agents for overactive bladder treatment,'' Journal of Pharmacology and Experimental Therapeutics, vol.325,no.3,pp.774-781,2008.
9. M. Kuipers, R. Smulders, W. Krauwinkel, and T. Hoon, '' Openlabel study of the safety and pharmacokintics of solifenacin in subjects with hepatic impairment,'' Journal of Pharmacological Sciences, vol. 102, no. 4, pp.405-412,2006.
10. E. Callegari, B. Malhaotra, P.J.Bungay et al., '' A comprehensive non-clincal evalution of the CNS penetration potential of antimuscarinic agents for the tretment of overactive bladder,'' British Journal of Clinical Pharmacology, vol.72, no2, pp.235-246,2011.
11. J.Macek, P.Ptacek, and J.Klima, '' Determination of solifenacin in human plasma by liquid chromatography-tandem mass spectrometry,'' Journal of Chromatography B; Analytical Tecnologies in the Biomedical and Life Sciences, vol.878,no .31,pp.3327-3330,2010.
12. H.N.Mistri, A.G.Jangid, A. Pudage, D.M.Rathod, and P.S. Shrivastav, '' Highly sensitive and rapid LC- ESI-MS/MS method for the simultaneous quantification of uroselective alpa1- blocker, alfuzosin and an antimuscarinic agent, solifenacin in human plasma, '' Journal of Chromatography B ; Analytical Technologies in the Biomedical and Life Sciences, vol. 876, no.2.pp.236-244,2008.
13. T. Yanagihara, T. Aoki, Y. Soeishi , T Iwastubo, and H. Kamimura,'' Determination of solifenacin succinate, a novel muscarinic receptor antagonist, and its major metabolite in rat plasma by semi-micro high perfomance liquid chromatography ,''Journal of Chromatography B; Analytical Technologies in the Biomedical and Life Sciences, vol. 859, no.2,pp.241-245,2007.
14. S.R.Krishna, B. M.Rao, and N.S.Rao, '' A validated rapid stability-indicating method for the determination of related substances in Solifenacin Succinate by ultra-fast liquid chromatography,'' Journal of Chromatographic Science, vol.48,no.10,pp.807-810,2010.
15. D.Desai, G. Patel, N.Shukala, and S.Rajput, '' Development and validation of stability –indicating HPLC method for solifenacin succinate; isolation and identification of major base degradation product, '' Acta Chromatograhica, vol.24,no 3, pp.399-418,2012.
16. B.V.Rami Reddy, B. Srinivasa Reddy, N.V.V.S.S. Raman, K. S.Reddy, and C. Rambabu, '' Development and validation of a specific stability indicating high performance liquid chromatograhic metods for related compounds and assay of solifenacin succinate ,'' Journal of Chemistry, vol .2013,Article ID 412353,10 pages ,2013.
17. D.J.Desai, G.Patel, D.R.A.Jain, and S.J. Rajput, '' Development and validation of stability-indicating HPTLC method of solifenacin succinate,'' Asian Journal of Pharmaceutical and Biological Research, vol.1, no.3,pp.310-316,2011.
18. A.M.Deegan, M.Cullen, M. Oelgemoller, K .Nolan, J. Tobin, and A. Morrissey, '' A SPE-LC-MS/MS method for the detection of low concentration of pharmacecutical in industrial waste streams,'' Analytical Letters, vol.44,no.17,pp.2808-2820,2011.
19. ICH Guidelines, '' Validation of analytical procedures; text and methodology,'' ICH Q2 (R1),2005.

Table 1: Assay of Solifenacine succinate

Commercial Formulation	Drug	Standard area	Sample Area	Label Claim (mg)	% Purity
Bispec10 mg	Solifenacine Succinate	1623856	1613568	10 mg	98.30

Validation Parameters

Accuracy and Recovery

Table 2: Accuracy of Solifenacin Succinate 10mg

Spiking level (%)	Solifenacin succinate added (µg/ml)	Recovered (µg/ml)	Recovery (%)
60	60.24	61.92	102.8
		61.74	102.5
		61.97	102.9
100	100.40	99.99	99.6
		99.59	99.2
		100.05	99.7
140	140.56	143.34	102.0
		143.19	101.9
		143.42	102.0

Table 3: Data analysis of Accuracy test

Results		
S.No		**Solifenacine Succinate 10mg tablet**
1	Mean	101.40
2	Standard Deviation	1.47
3	Number of sample (n)	9.00
4	95% Confidence level	0.96
5	95%Confidence limit (Lower)	100.44
6	95%Confidence limit (upper)	102.36

Solifenacin Succinate 10mg accuracy was study done in 60%,100%,and 140%.Recovery and percentage purity of Solifenacin succinate were found in the range of 99.23-99.50% respectively.

Linearity

Table 4: Linearity of Solifenacin succinate

S.NO	Solifenacin Succinate	
	Conc (mcg/mL)	Mean area
1	50.200	815274
2	80.320	1275226
3	100.400	1626599
4	120.480	1927650
5	150.600	2422780

Precision

Table 5: Intermediate precision of Solifenacin succinate

Analyst	%Assay 10mg
1	101.5
2	102.0
3	102.9
4	101.9
5	101.9
6	100.4
Mean	101.8
%RSD	0.80

Intermediate precision was carried in Solifenacin succinate to determine repeatability of method development. With the same solution precision was carried out next day also. % RSD was not more than 3 as per ICH guidelines.

Robustness

Table 6: Robustness of Solifenacin Succinate

S.No	Changed Method parameter	Actual condition	Assay%L.A 10mg tablet
1	Flow rate 0.8ml/min		100.0
2		Flow rate 1.0ml/min	101.5
3	Flow rate1.2ml/min		101.1
4	Column temperature 25*C		100.6
5		Column temperature 30*C	101.5
6	Column temperature 35*C		101.1
Average			100.97
%RSD			0.573

Robustness study was done by changing the Column temperature, flow rate in both the drug. % RSD was not more than 2 as per ICH guidelines.

Table 7: Limit of detection (LOD) and Limit of quantification (LOQ) of Solifenacine Succinate

S.No	Parameters	Solifenacine Succinate
1.	LOD	3.14
2.	LOQ	14.16

Limit of detection was carried out in Solifenacinesuccinate the value are found to be 3.14 respectively. Limit of quantification was carried out in Solifenacine succinate the value are found to be 14.16 respectively.

Table.8.Acceptance limit for Validation parameters

S.No	Validation Parameter	Acceptance Limit
1	System Precision	RSD for replicate injection NMT 2.0%
		Tailing factor NMT 2.0
		Number of theoretical plates NLT 2000
2	Method Precision I)Repeatability II) Ruggedness	 i)%RSD of 6 sample results :NMT 2.0% ii)%RSD of 12 sample results : NMT 3.0%
3	Accuracy (Recovery)	NLT 95% and NMT 105 105%
4	Linearity and Range	Correlation Coeffivient (R2) NLT 0.995
5	Identification and specificity	For identification :Ratio of retention time NLT 0.95 and NMT 1.05
		No interference should be apparent

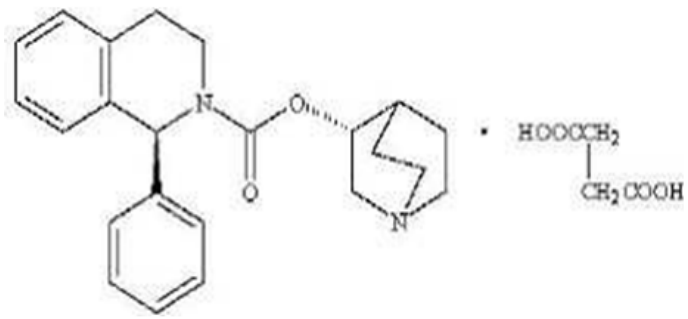

Figure 1: Chemical structure of Solifenacine succinate

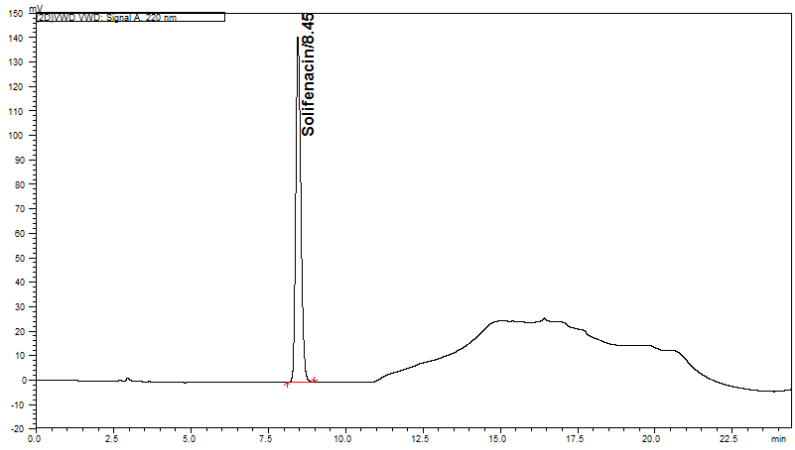

Figure 2: Chromatogram of Solifenacine succinate

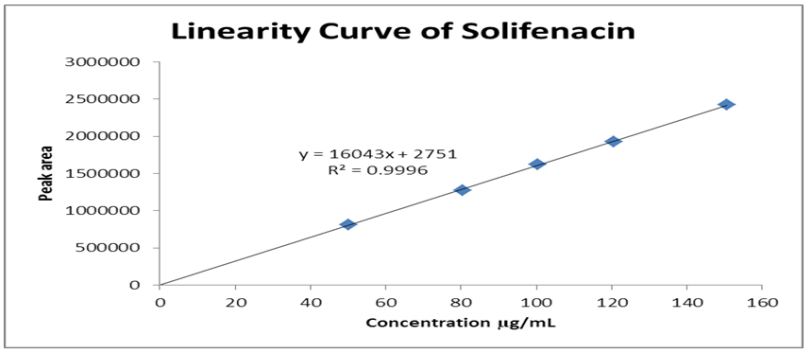

Figure: 3 Linearity of Solifenacin succinate

Linearity of Solifenacin Succinate were found to be (50.200µg/ml to 150.600µg/ml) respectively. The % RSD of Solifenacin succinate were found to be 0.9996 were not more than 1as per ICH guidelines.

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RESEARCH ARTICLE

Application of Bidirectional DC Converter for Battery Energy System

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ABSTRACT

The quasi-Z-source (qZS) converter is used to create a new bidirectional dc-dc converter. It functions as a traditional qZS full-bridge structure with a synchronous voltage doubler rectifier during battery draining. It functions as a half-bridge structure with a synchronous full-bridge rectifier and LC-filter during battery charging. For switching between the two modes, a relay is utilized. The operating concept is described, as well as design recommendations. For analysis of constant regulation parameters and efficiency tests in the input voltage area compatible with an eight-cell LiFePO4 battery, a prototype with a nominal power of 300 W was utilized. In two control scenarios: dc-bus signalling and direct reference specified by a master controller via a data channel, a closed loop control structure for the converter application in dc microgrids is described and verified.

Keywords: quasi-Z-source converter, bidirectional converter, dc-dc converter, battery energy storage system.

INTRODUCTION

The interest in energy and system output in the designing area has grown in recent years across the world. According to estimates, residential and commercial buildings account for almost 40% of total output use in the European Union and the United States [1]. This industry is growing at a rapid pace, resulting in increased energy utilization and carbon emissions [2]. As a result, in the construction industry, energy conservation and the use of non conventional energy sources are critical steps that must be taken to decrease energy requirement and greenhouse gas emissions [3], [4]. Power electronics plays a significant role in this context, allowing for efficient electric power conversion and simple integration and management of renewable energy sources and energy storages, resulting in

the best possible power system performance [5], [6]. Engineering scientists have begun to introduce power electronics in brand new applications, such as the Electronic Power Distribution System (EPDS), also known as Active Distribution Network or Smart Grid [7]-[10], thanks to rapid advances in semiconductor and packaging technologies, as well as the development of new power converter topologies. Such ideas may be used for a single building [11]-[14] or a district [15]-[18] depending on the power scale, allowing for greater proportions of distributed energy production and storage, demand-side efficiency, and energy trading activities. One of the most common conceptual approaches to the EPDS for residential structures is shown in Figure 1 [19]. Due to the absence of grid-tied inverters, which are integral parts of the interface converters for traditional AC power distribution systems, the interconnection and interaction of distributed generators (DGs), energy storages (DESs), and loads is realised through the high-voltage dc-bus, which results in simplified power processing and increased power conversion efficiency [20], [21].

Renewable energy sources cover a substantial portion of the energy demand of zero-energy and resource-efficient buildings [22]. The major contributions to the energy performance of the domestic power system are energy storage technologies [22]-[24]. The proposed EPDS includes an auxiliary AC bus to guarantee compatibility with presently prevalent AC loads, which may be completely removed in the future with the adoption of a dc supply standard for consumer loads [25]-[27]. The plug-and-play capability of the dc linked EPDS, as well as the simpler interaction of the DGs and DESs, are major advantages. It offers a one-of-a-kind chance to use adaptable modular solutions, lowering weight and installation area, which is becoming more essential in residential construction. Because of the module-level maximum power point tracking, micro converters used in rooftop solar systems provide for greater system design flexibility, module-level monitoring and diagnostics, simpler installation, and higher energy output from the PV module [28], [29]. In contrast to conventional PV string inverters, the PV microconverters are linked in parallel at the HV dc-bus, making power scaling up more simpler and quicker. The home battery energy storage system (RBESS) [30] may use the similar modularity concept. A RBESS may be tailored to the requirements of a specific family by cascading tiny units in parallel [31]. RBESS between power and capacity is typically in the range of 0.25 W/(W•h) to 0.75 W/(W•h) [32], with 0.25 W/(W•h) providing the best economic performance [33]. This research focuses on LiFePO4 battery dc-bus interfacing, which is beneficial in residential applications owing to their extended lifespan, durability, and better safety [34]. The developed converter is intended for use with a 24 V LiFePO4 battery with a 1.2 kWh capacity and a nominal output of 300 W, resulting in a ratio of 0.25 W/(W•h), which is suitable for modular RBESS.

It may be comparable to commercial AC-coupled batteries as the Enphase AC battery, SOLARWATT My Reserve 500, and Solar World SunPac LiOn 2. Furthermore, the developed converter could be used for battery charging of small electric vehicles (electric bicycles, scooters, power assist wheelchairs, and so on) as well as the implementation of some service functions (for example, battery "refresh"), which are critical in the case of some battery technologies used in such vehicles. This article expands on the concepts given in [36], in which it was shown that the topology from [29] could be used to accomplish reverse energy transfer via a variety of control techniques, with topology reconfiguration by a relay demonstrating the greatest performance. Only feasible options for reverse energy transfer in the galvanically isolated quasi-Z-source full-bridge dc-dc converter were presented in previous findings [30]. As a consequence, the following are the contributions of this article to the findings presented in [25] and [15]. First, a closed-loop control theory appropriate for use in dc microgrids is developed based on a thorough examination of operating modes (charging and discharging) and a thorough assessment of their experimental performance. Second, the converter is experimentally justified for dc microgrid applications utilizing the same control system for both centralized and decentralized control, with excellent dynamics obtained via feed forward control. Both operational modes of this control system use the same modulator. Third, the modular RBESS technology is examined in the context of small residential dc microgrids, with the optimum size and power-to-capacity ratio established using existing research and industry solutions. Overall, this work adds to the fields of galvanically isolated impedance-source dc-dc converters and reconfigurable converters with topology morphing control. This article makes a practical

contribution by proposing an open hardware solution for modular RBESSs that meets the requirements of the fast growing industry of technologies for near-zero energy buildings.

Bidirectional quasi-Z-source DC-DC converter

A galvanically isolated impedance-source dc-dc converter is proposed as a versatile power conversion technique for applications needing a wide range of input voltage and load management. Many of the important characteristics of the topology and its many variations have been demonstrated in renewable and alternative energy systems, where they have demonstrated such important characteristics as continuous input current, increased reliability due to inherent short- and open-circuit immunity, and high power conversion efficiency over a broad gain range [27]. Because ISCs combine the main features of voltage- and current-source converters, they may be regarded a new type of power converters that perform both buck and boost functions in a single switching stage, according to the findings of the technical research [30]. With its relative simplicity and high control flexibility, the quasi-Z-source full-bridge dc-dc converter (qZSC) [20] from the family of galvanically isolated ISC [29], which already includes more than 30 distinct topologies [24], appears to be the most promising due to its relatively simple structure. Because of the unique features of the quasi-Z-source (qZS) network, the full-bridge inverter could handle all possible switching states; as a result, multimode control (MMC) could be implemented, allowing for a ten-fold increase in input voltage regulation range [29]. Any shoot-through control method may be used in conjunction with phase-shift modulation [29], asymmetrical pulse-width modulation or variable frequency control to accomplish the MMC in the buck mode. The series resonant tank, which may be comprised of additional components [18] or fully integrated into the secondary side of the converter is often employed to assist the qZSC's ability to operate across a wide input voltage and load range. In order to reduce conduction losses in qZSC converters, it is highly recommended that they be connected to a synchronous qZS network, which may increase the overall efficiency of the converter by more than 2 percent depending on the operating point [20].

A power MOSFET may also be used to replace the qZS diode in order to relieve some of the converter's instability problems while operating in the discontinuous conduction mode of operation (DCM). The behaviour of MOSFETs in the third quadrant allows the input current to go negative while still maintaining stable operation under low load conditions, which is advantageous. The performance of the qZSC may be improved even further by substituting controlled switches for the rectifier diodes on the secondary (high-voltage) side of the converter. Among the benefits are not just improved efficiency as a result of reduced conduction losses [21], but also the ability to control bidirectional power flow without requiring any other significant topological modifications. In reverse mode, the qZS-network behaves as a low pass LC-filter, which ensures that the output voltage remains stable within a specified regulatory range, as described in [20]. Despite the fact that the first bidirectional ISC concepts were proposed more than five years ago, their viability has been called into question due to the complexity of the power circuit (two qZS-networks are used) [28] or the efficiency and controllability issues that could arise from the use of the power switches' integrated antiparallel diodes as uncontrolled rectifiers ISC's reverse power flow control capabilities have recently been enhanced with the addition of two new techniques that take advantage of the resonant characteristics of the topology to ensure high efficiency in the power conversion process as well as linearity of control variables with little reliance on the operating power [28]. It is discussed in this article as a possible architecture for the modular RBESS's power electronic interface, namely the novel bidirectional qZS dc-dc converter shown in Fig. 2. In the battery discharge (forward) mode, the proposed converter works as a full-synchronous step-up impedance-source converter with shoot-through pulse-width modulation (PWM) (ST-PWM). In battery charging (reverse) mode, the qZS-network is reconfigured into an LC-filter, and the converter operates as a full-synchronous voltage-source step-down dc-dc converter with an appropriately adjusted PWM. The proposed converter, in contrast to the previous approach [32], does not have resonant properties. It has previously been proposed to use resonant features to either extend the voltage control range [29], which is not required in this application, or to use an inefficient alternate reverse energy transfer method [30]. Furthermore, whether or not the converter is resonant has no effect on whether form of reverse energy transfer is more efficient in terms of efficiency. Parallel to this, resonant operation places constraints on converter design by limiting the maximum quality factor Q 1, which in turn restricts the range of

transformer leakage inductance values and, as a result, influences the selection of magnetising inductances. As a consequence, due to the relatively high shoot-through current required by the qZS network, resonant operation does not result in soft-switching of the front-end switches during operation. As a consequence, preventing resonance in converters is less difficult to design and maintain than before.

Principle of operation the converter proposed converter

Drawings and oscillograms of current directions indicate that the positive sign of the current corresponds to the directions illustrated in Fig. 2. More significantly, the modulation is the same for both modes, which makes its implementation easier since it is consistent. It is used in the battery charging mode as a component of a dissipative voltage overshoot clamping circuit, whereas it dissipates little power in the battery discharging mode because the qZS-network has inherent dc rail voltage clamping [30] and thus dissipates little power during the battery charging mode. The converter operates in this mode in the same way as the full-bridge qZSC [31]. When a high voltage (HV) side transformer winding is energised, parasitic oscillations may develop. The ST-PWM is achieved via the symmetrical overlap of active states in order to improve transformer utilization while simultaneously reducing losses. While the nature of voltage clamping is more complicated, it is shown in Fig. 5 via the use of simplifications to illustrate the concept.

Simulation results

The Matlab/Simulink implementation of the suggested system is shown in Fig. 3. Fig. 4 indicates the state of charge, Fig. 5 indicate the output current, Fig. 6 indicate the battery voltage, Fig. 7 indicate the transformer voltage, Fig. 8 indicate the output voltage and Fig. 9 is output current.

CONCLUSION

This article describes a bidirectional galvanically isolated dc-dc structure based on a quasi-Z-source. The idea surpasses any current rival in the area of impedance-source converters. It's designed for home battery energy storage systems that are modular. The proposed control system includes on-the-fly converter topology modification to provide acceptable efficiency in any of the energy transfer areas. For the nominal battery voltage, the efficiency is in the region of 95-96 percent, while the range of 20 V to 30 V is covered for the optimum battery usage. In the battery draining mode, the peak efficiency is 97.2 percent, however as the battery voltage lowers, the efficiency diminishes. In the battery charging mode, peak efficiency is lower, but efficiency is less reliant on battery voltage. For modular home BESS with modules cascaded in parallel, the new converter was designed, evaluated, confirmed experimentally, and justified. This method has been verified using MATLAB simulation and testing, and the results indicate that the proposed technique achieves its goal.

REFERENCES

1. P. Nejat, F. Jomehzadeh, M. M. Taheri, M. Gohari, and M. Z. Abd. Majid, “A global review of energy consumption, CO2 emissions and policy in the residential sector (with an overview of the top ten CO2 emitting countries),” *Renewable and Sustainable Energy Reviews*, vol. 43, pp. 843–862, Mar. 2015.
2. Allouhi, Y. El Fouih, T. Kousksou, A. Jamil, Y. Zeraouli, and Y. Mourad, “Energy consumption and efficiency in buildings: current status and future trends,” *J. of Cleaner Prod.*, vol. 109, pp. 118–130, 2015.
3. P. Moran, J. Goggins, and M. Hajdukiewicz, “Super-insulate or use renewable technology? Life cycle cost, energy and global warming potential analysis of nearly zero energy buildings (NZEB) in a temperate oceanic climate,” *Energy and Buildings*, vol. 139, pp. 590–607, 2017.
4. N. Soares et al., “A review on current advances in the energy and environmental performance of buildings towards a more sustainable built environment,” *Renewable and Sustainable Energy Reviews*, vol. 77, pp. 845–860, Sep. 2017.

5. F. Blaabjerg, Zhe Chen and S. B. Kjaer, "Power electronics as efficient interface in dispersed power generation systems," *IEEE Trans. Power Electron.*, vol. 19, no. 5, pp. 1184-1194, Sept. 2004.
6. F. Blaabjerg, K. Ma and Y. Yang, "Power electronics - The key technology for Renewable Energy Systems," *Proc. 9th Int. Conf. Ecological Veh. and Renewable Energies*, Monte-Carlo, 2014, 11 pp.
7. Boroyevich, D.; Burgos, R.; Arnedo, L.; Fei Wang, "Synthesis and Integration of Future Electronic Power Distribution Systems", *Proc. Power Conversion Conference (PCC'07)*, pp. K-1-K-8, 2-5 April 2007.
8. Ajaja, A., "Reinventing electric distribution", *IEEE Potentials*, vol.29, no. 1, pp.29-31, Jan.-Feb. 2010.
9. S. Chowdhury, S.P. Chowdhury and P. Crossley, "Microgrids and Active Distribution Networks," ISBN: 978-1-84919-014-5, IET, 2009.
10. D. Boroyevich, I. Cvetkovic, R. Burgos and D. Dong, "Intergrid: A Future Electronic Energy Network?," *EEE J. Emerg. Selt. Topics Power Electron.*, vol. 1, no. 3, pp. 127-138, Sept. 2013.
11. G. Comodi et al., "Multi-apartment residential microgrid with electrical and thermal storage devices: Experimental analysis and simulation of energy management strategies," *Appl. Energy*, vol. 137, pp. 854–866, Jan. 2015.
12. R. Atia and N. Yamada, "Sizing and Analysis of Renewable Energy and Battery Systems in Residential Microgrids," *IEEE Trans. Smart Grid*, vol. 7, no. 3, pp. 1204–1213, May 2016.
13. D. Zhang, N. Shah, L. G. Papageorgiou, "Efficient energy consumption and operation management in a smart building with microgrid," *Energy Conversion and Manage.*, vol. 74, pp. 209–222, Oct. 2013.
14. X. Guan, Z. Xu, Q. S. Jia, "Energy-Efficient Buildings Facilitated by Microgrid,"*IEEE Trans. Smart Grid*, vol.1, no.3, pp.243-252, Dec. 2010.
15. M. P. Fanti, A. M. Mangini, M. Roccotelli, W. Ukovich and S. Pizzuti, "A control strategy for district energy management," *Proc. IEEE Int. Conf. Automat. Science and Eng.*, Gothenburg, 2015, pp. 432-437.
16. M. P. Fanti, A. M. Mangini, M. Roccotelli, and W. Ukovich, "District Microgrid Management Integrated with Renewable Energy Sources, Energy Storage Systems and Electric Vehicles," *IFAC-Papers OnLine*, vol. 50, no. 1, pp. 10015–10020, Jul. 2017.
17. G. M. Kopanos, M. C. Georgiadis, and E. N. Pistikopoulos, "Energy production planning of a network of micro combined heat and power generators," *Appl. Energy*, vol. 102, pp. 1522–1534, Feb. 2013.
18. Reddy, Ch Rami, and K. Harinadha Reddy. "Islanding detection for inverter based distributed generation with Low frequency current harmonic injection through Q controller and ROCOF analysis." *Journal of electrical systems* 14, no. 2 (2018): 179-191.
19. Ch, Rami Reddy, and K. Harinadha Reddy. "An efficient passive islanding detection method for integrated DG system with zero NDZ." *International Journal of Renewable Energy Research (IJRER)* 8, no. 4 (2018): 1994-2002.
20. Reddy, Ch Rami, K. Harinadha Reddy, and K. Venkata Siva Reddy. "Recognition of islanding data for multiple distributed generation systems with ROCOF shore up analysis." In *Smart Intelligent Computing and Applications*, pp. 547-558. Springer, Singapore, 2019.
21. Reddy, Ch Rami, and K. Harinadha Reddy. "A new passive islanding detection technique for integrated distributed generation system using rate of change of regulator voltage over reactive power at balanced islanding." *Journal of Electrical Engineering & Technology* 14, no. 2 (2019): 527-534.
22. Reddy, Ch Rami, and K. Harinadha Reddy. "Islanding detection techniques for grid integrated distributed generation-A review." *International Journal of Renewable Energy Research* 9, no. 2 (2019): 960-977.
23. GOUD, B. SRIKANTH, and Ch Rami Reddy. "Essentials for grid integration of hybrid renewable energy systems: a brief review." *International Journal of Renewable Energy Research (IJRER)* 10, no. 2 (2020): 813-830.
24. Suresh, K., P. Anusha, Sk Najma, B. I. Rajkumar, Ch Rami Reddy, and B. Prasanna Lakshmi. "A passive islanding detection method for hybrid distributed generation system under balanced islanding." *Indonesian Journal of Electrical Engineering and Computer Science* 14, no. 1 (2019): 9-19.
25. Reddy, Ch Rami, and K. Harinadha Reddy. "Passive islanding detection technique for integrated distributed generation at zero power balanced islanding." *International Journal of Integrated Engineering* 11, no. 6 (2019): 126-137.

26. Reddy, Jetty Rajesh, Alagappan Pandian, and Chilakala Rami Reddy. "An efficient learning based RFMFA technique for islanding detection scheme in distributed generation systems." *Applied Soft Computing* 96 (2020): 106638.
27. Goud, B. Srikanth, B. Loveswara Rao, and Ch Rami Reddy. "An intelligent technique for optimal power quality reinforcement in a grid-connected HRES system: EVORFA technique." *International Journal of Numerical Modelling: Electronic Networks, Devices and Fields* 34, no. 2 (2021): e2833.
28. Raju, S. Govinda, K. Harinadha Reddy, and Ch Reddy. "Islanding Detection Parameters for Integrated Distributed Generation." *Recent Advances in Electrical & Electronic Engineering (Formerly Recent Patents on Electrical & Electronic Engineering)* 14, no. 2 (2021): 131-143.
29. Reddy, Ch Rami, K. Harinadha Reddy, B. Srikanth Goud, and B. Pakkiraiah. "A Deep learning approach for Islanding Detection of Integrated DG with CWT and CNN." In *2021 International Conference on Sustainable Energy and Future Electric Transportation (SEFET)*, pp. 1-7. IEEE, 2021.
30. Thumu, Raghu, Kadapa Harinadha Reddy, and Chilakala Rami Reddy. "Unified power flow controller in grid-connected hybrid renewable energy system for power flow control using an elitist control strategy." *Transactions of the Institute of Measurement and Control* 43, no. 1 (2021): 228-247.
31. Goud, B. Srikanth, P. Srinivasa Varma, B. Loveswara Rao, M. Sai Krishna Reddy, A. Pandian, and Ch Rami Reddy. "Cuckoo Search Optimization based MPPT for Integrated DFIG-Wind Energy System." In *2020 International Conference on Decision Aid Sciences and Application (DASA)*, pp. 636-639. IEEE, 2020.
32. Goud, B. Srikanth, R. Rekha, M. R. L. Jyostna, S. Sarala, B. Loveswara Rao, and Ch Rami Reddy. "Energy Management and Power Quality Improvement in HRES Grid-Connected System." In *2020 FORTEI-International Conference on Electrical Engineering (FORTEI-ICEE)*, pp. 174-178. IEEE, 2020.
33. Reddy, Ch Rami, K. Naresh, P. Umapathi Reddy, and P. Sujatha. "Control of DFIG Based Wind Turbine with Hybrid Controllers." *International Journal of Renewable Energy Research (IJRER)* 10, no. 3 (2020): 1488-1500.

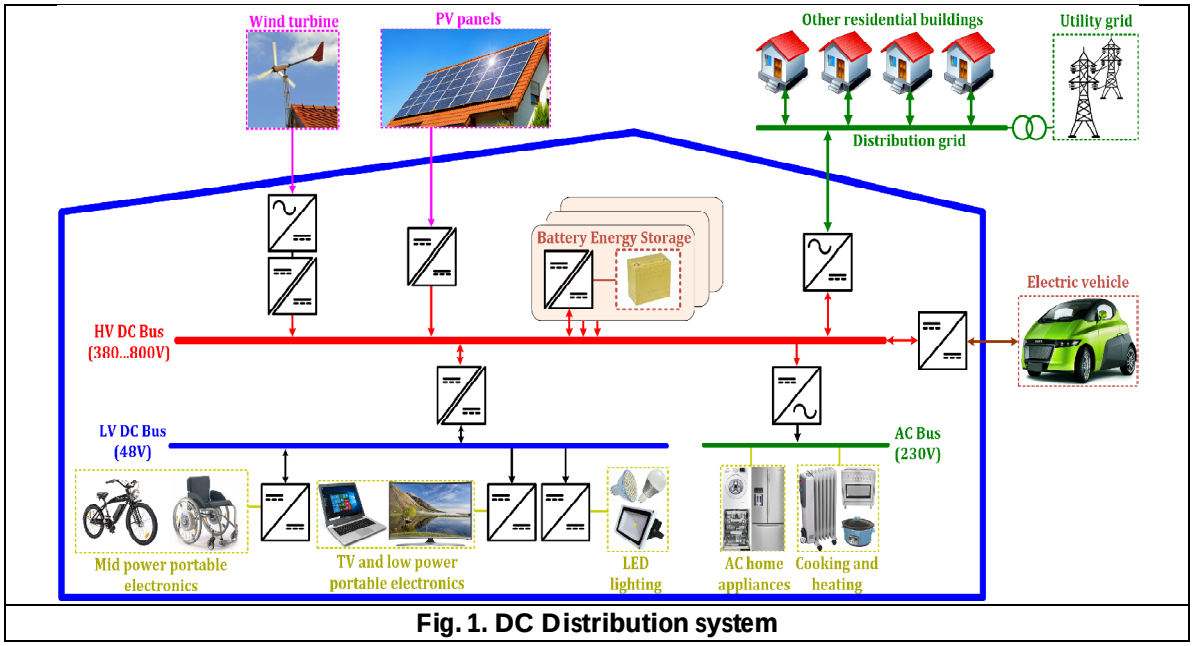

Fig. 1. DC Distribution system

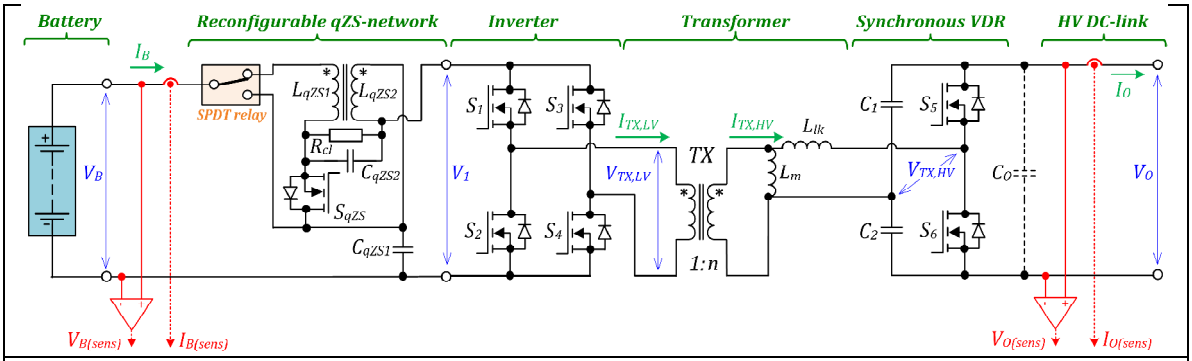

Fig 2: Quasi-Z-source dc-dc converter with the reconfigurable quasi-Z-source system

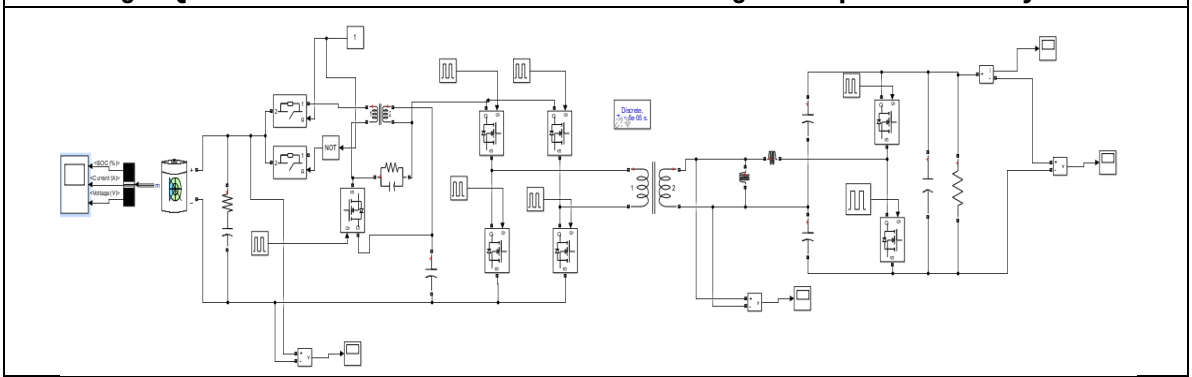

Fig 3: Simulation circuit

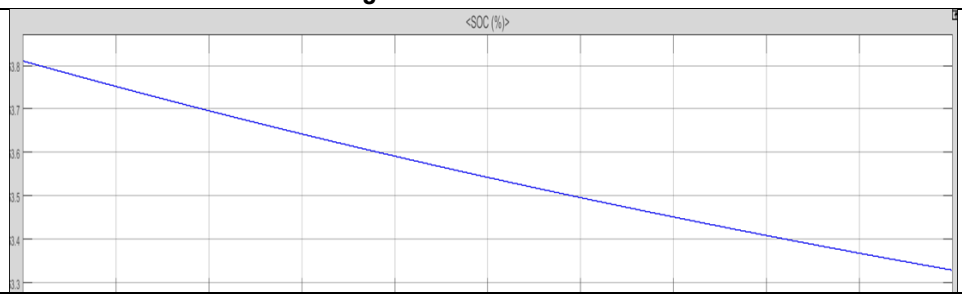

Fig 4: State of charge

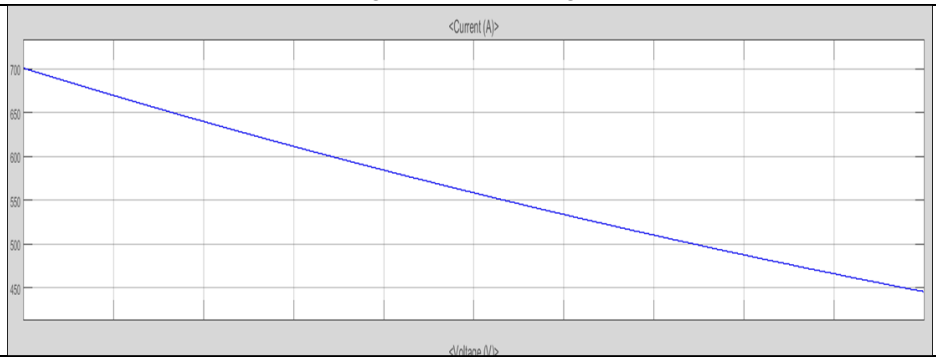

Fig 5: Output current

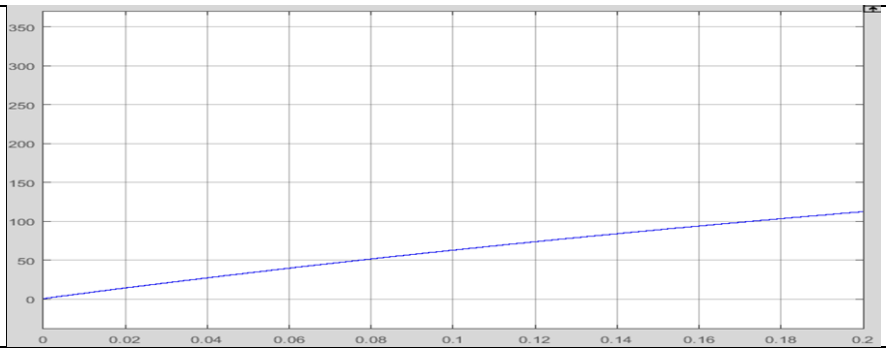

Fig 6: Battery voltage

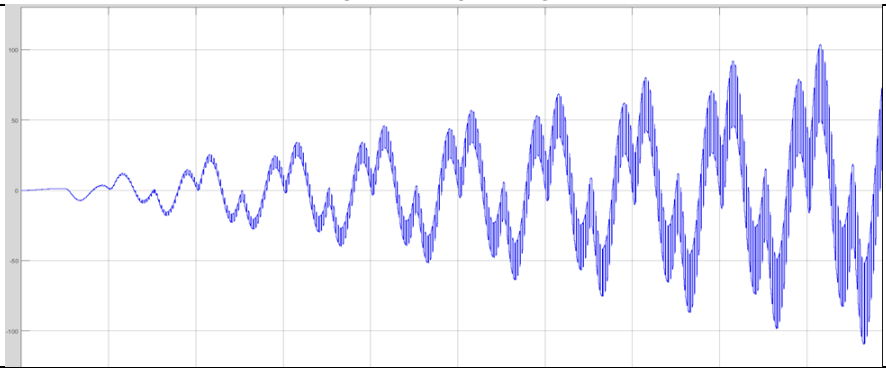

Fig 7: (a) Transformer voltage

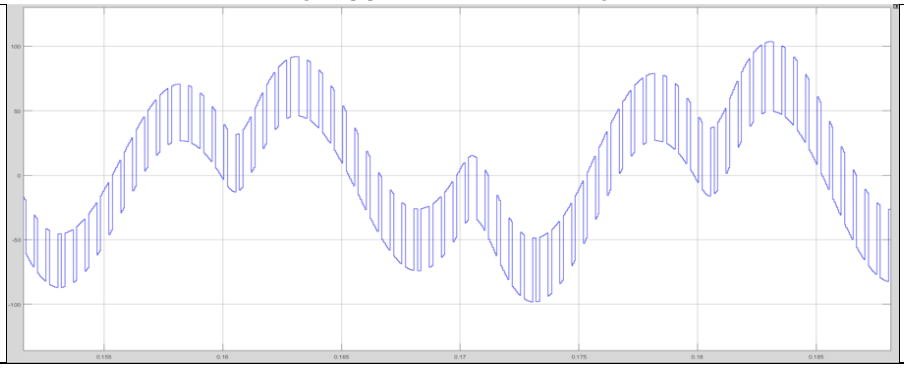

Fig 7: (b) Transformer voltage

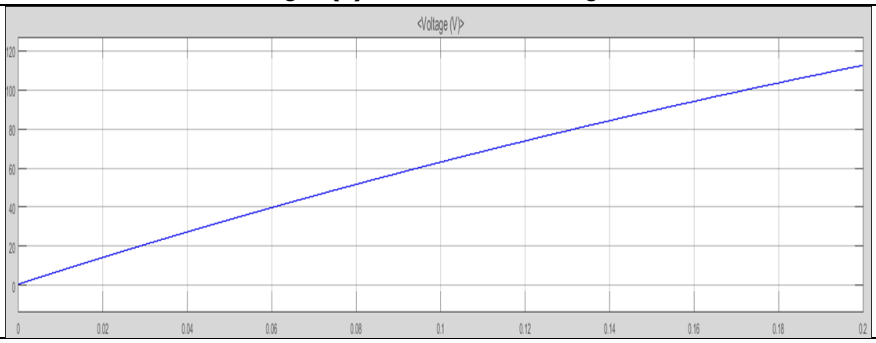

Fig 8: Output voltage

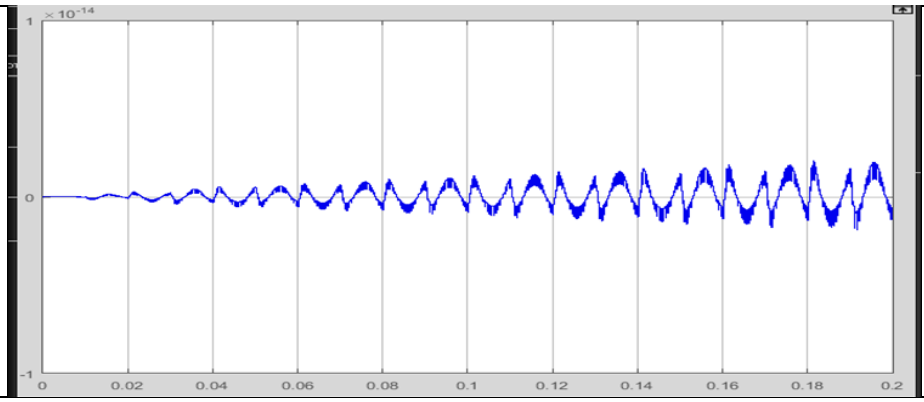

Fig 9: Output current

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RESEARCH ARTICLE

3Φ Transformerless Shunt Active Power Filter for Harmonic Compensation

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ABSTRACT

Power quality decline has an adverse economic impact on utilities and their customers. One of the most well-known power quality challenges is harmonics in current and voltage, which can be handled by using a Hybrid Series Active Power Filter (HSAPF). To make the HSAPF more robust and reliable, a novel controller design based on sliding mode controller-2 is proposed in this study. In this research, an accurate averaged model of three-phase HSAPF is also developed. The resilient HSAPF design concept has been validated by simulation and the results have been discussed.

Keywords: Power Quality, Active Power Filter, Harmonics, controller

INTRODUCTION

Electricity demand is steadily expanding in the modern industrial world, from residential utilities to commercial businesses. Integrating distributed energy resources such as solar photovoltaic systems, wind energy conversion systems, fuel cells, distributed power production systems, and storage devices enhances reliability and power quality while lowering losses of power distribution or transmission networks. The massive expansion in the use of non-linear loads in recent years has resulted in a slew of power quality difficulties on the electrical grid, including excessive current harmonics, voltage distortion, and low power factor, to name a few. Harmonic currents are injected into the AC power lines as a result of the development of non-linear loads in the system. This distorted supply voltage and current causes some protective systems to fail, transformers and motors to burn out, and cables to overheat. As a result, Passive power filters have traditionally been employed as a compensation device to correct for distortion caused by constant non-linear loads. With a simple design and inexpensive cost, these filters [2] are designed to provide a low impedance channel for harmonics while preserving high power quality. Passive filters, on the other hand, have drawbacks such as mistuning, resonance, reliance on power supply system circumstances, and

large passive component values, all of which result in bulky implementations. These filters are the most popular because they effectively minimize current distortion and reactive power generated by non-linear loads. However, they are typically costly and have high operational losses. Peng et al. [5] introduced a novel HAPF topology- III in 1988 to overcome these drawbacks and improve compensation performance while lowering the cost of the APFs. In this topology, the APF is connected in series with the source as well as the non-linear load, while the PPF is connected in parallel with the load and acts as a PFC capacitor is proposed. This topology [6] drew a lot of interest since it can withstand high load currents and acts as a harmonic isolator between the source and the non-linear load.
The control method is critical for improving HSAPF's performance. Many publications on hybrid power filters have already offered improved approaches for reducing current harmonics caused by non-linear loads. For a hybrid power filter, [7] proposes a linear feedback-feed forward controller. However, because the dynamic model of the HSAPF system comprises multiplication terms of control inputs and state variables, this controller is difficult to get both steady-state and transient state performances with the linear control method. A sliding mode controller is provided in [8] due to the non-linear properties of HSAPF. Furthermore, by decoupling the system into distinct subsystems of lesser dimension, this sliding mode control reduces the complexity of feedback control design. The application of sliding mode control can be found in the domains of power electronic switching devices due to these qualities. The principle of sliding mode control is to use discontinuous control to impose sliding mode motion in a designated switching surface of the system state space. The switching surfaces should be chosen so that sliding motion maintains the desired motion dynamics according to a set of performance criteria. For linear systems, traditional control methods such as Linear-quadratic regulator (LQR) [9] or Linear quadratic Gaussian (LQG) servo controller [10].

The discontinuous control must then be chosen in such a way that all states outside of the discontinuity surface must reach it in a finite amount of time. As a result, the system enters a sliding mode down the surface and follows the inte nded system dynamics. Chattering is the most difficult aspect of implementing the classic sliding mode control mechanism in hardware. Chattering is a type of oscillation with a finite frequency and amplitude that is restricted. The chattering is dangerous because the system lacks control accuracy, moving mechanical elements wear out quickly, and electrical power circuits lose a lot of heat. The switching frequency in sliding mode control should be high enough to make the controller more robust, stable, and free of chattering, as chattering decreases as the switching frequency of the system increases. Increased switching frequency is a natural technique to prevent chattering when using a sliding mode controller in power converter systems, such as HSAPF. However, due to certain constraints in switching frequency for losses in power converters, it is not practicable in the case of power converters, resulting in chattering. As a result, the chattering problem cannot be blamed on the implementation of sliding mode because it is primarily caused by switching constraints. When the relative degree of the system with actuators or sensors is two, the chattering exponentially decreases to zero, as illustrated in [11]. The HSAPF system has a relative degree of two. This study work presented a novel controller, the sliding mode controller-2, as a result of the relative degree of the HSAPF system and the barriers in the classical sliding mode controller. This proposed controller reduced chattering and improved HSAPF performance. This controller is brand new for this HSAPF system topology. The carrier based PWM (CBPWM) for HSAPF architecture is the subject of a new research publication [12]. However, in the majority of real-world scenarios, the CBPWM-based HSAPF may not be completely measurable. In the case of CBPWM, power system perturbations are not taken into account, and the presence of a temporal delay at the reference tracking point causes the total system to respond slowly. Section II describes the schematic of the system topology of the three-phase HSAPF model. The averaged modelling of the HSAPF system is depicted in Section III. Section IV reveals the HSAPF controller design. Section-V shows the simulation results for harmonic compensation with HSAPF. This work's conclusions are presented in Section VI.

Description of System Topology Schematic and HSAPF Model Hardware Modules

Figures 1 shows a schematic diagram of a hybrid series active power filter (HSAPF). This HSAPF topology is made up of a series connected active power filter (SAPF) and a shunt connected passive power filter (PPF). The PPF is linked in parallel with the load. The PPF is made up of a fifth and seventh tuned LC filter of rating (Lpf = 1.9mH and

Cpf = 80F) for harmonic current compensation on the load side. To ensure galvanic isolation, the SAPF is connected in series with the source via a matching transformer with a turn ratio of 1:2. The high frequency LC filter is used to remove high frequency switching ripples from the inverter's compensating voltage. The HSAPF is controlled by a controller-based algorithm that has been implemented.

HSAPF Average Modelling

The schematic diagram of the 3-phase HSAPF control and power circuit is shown in Fig. 1. The SAPF is made up of a voltage source inverter that is linked to the grid via an LC filter and a three phase linear transformer. The inductors series resistance is ignored.

$$\frac{di_{cd}}{dt} = \frac{V_{cd}}{L_f} + wi_{cq} - \frac{u_d V_{dc}}{L_f} \quad (1)$$

$$\frac{di_{cq}}{dt} = \frac{V_{cq}}{L_f} - wi_{cd} - \frac{u_q V_{dc}}{L_f} \quad (2)$$

$$\frac{dV_{cd}}{dt} - wV_{cq} - \frac{i_{cd}}{C_f} + \frac{i_{sd}}{C_f} \quad (3)$$

$$\frac{dV_{cq}}{dt} = wV_{cd} \quad \frac{i_{cq}}{C_f} \mid \frac{i_{sq}}{C_f} \quad (4)$$

$$\frac{dV_{dc}}{dt} - \frac{3}{2C_{dc}} (u_d i_{cd} + u_q i_{cq}) \quad (5)$$

Where u_a, u_b, u_c is the duty cycle (δ) of the inverter legs in a switching period, and V_{ca}, V_{cb}, V_{cc} is the output voltage of a three-phase series active filter, as shown in Fig. 2, and I_{ca} is the three-phase active filter current output, V_{aN}, V_{bN}, V_{cN} is the three-phase phase voltage, I_{sa}, I_{sb}, I_{sc} is the 3Φ source current, and V_{nN} is the neutral voltage. The whole averaged model [13] of the inverter in three phases is obtained by averaging the inverter legs in the circuit diagram, as shown in Fig. 3. The dynamic model of HSAPF under SRF can be expressed by the differential equations shown in this circuit diagram.

Where V_{cd} and V_{cq} are the d-q axis compensating voltages, u_d and u_q are the d-q axis duty ratios, and is the source voltage's angular frequency. The HSAPF system model can be defined as follows to aid controller design:

$$\begin{cases} x^{\cdot} = f(x) + g(x)\,u \\ y = h(x) \end{cases} \quad (6)$$

Where

$x = [i_{cd}, i_{cq}, V_{cd}, V_{cq}, V_{dc}]^T$ state vector,

vector $u = [u_d, u_q]^T$ system control variables,

vector $y = [y_1, y_2]^T = [V_{cd}, V_{cq}]^T$ system outputs.

It should be noted that the achieved Multi-Input Multi-Output (MIMO) system is non-linear due to the presence of state variable and control variable multiplication terms. Furthermore, the state variables are inextricably linked to one another.

These two difficulties can be precisely controlled by the design of a sliding mode controller, which examines the relationship between the control variables and the system outputs openly.

Control System Development

The reference compensation voltage of the HSAPF system using the hybrid control approach-based SRF method is expressed as follows:

$$V_c^* = KI_{sh} - V_{Lh} \qquad (7)$$

Figure 2 depicts the generation of a reference compensating signal using the combined source current detection and load voltage scheme[14]. Because the additional fundamental components are added to the harmonic components. As a result, the reference compensating voltages are also written as:

HSAPF Sliding Mode Controller Design Proposal

This section describes the synthesis of a sliding mode controller based on the HSAPF system's averaged model. We differentiate the compensating voltage with respect to time using system model (6) until the control variables u_d and u_q appear explicitly. The control signal in this proposed control approach satisfies all of the above conditions, causing the state trajectories to be moved towards the switching surface. As a result of using this proposed controller, the HSAPF system achieves fast response, good robustness, and effective throwaway disturbances.

SIMULATION RESULTS

MATLAB/Simulink software is used to test the reference generation approach (HSRF method) with the switching pattern generation scheme (i.e. sliding mode controller-2) of the HSAPF system shown in Fig. 2. A three-phase source voltage is applied to a non-linear load with a harmonic voltage. This voltage-producing nonlinear load is made up of a three-phase diode bridge rectifier feeding an RL-load. Harmonic distortion occurs in both the source current and the load voltage as a result of this type of non-linear load. Power quality disturbances are caused by harmonic contamination. As a result, HSAPF can eliminate power quality disturbances. MATLAB simulation results for steady-state source voltage Vs, load current IL, source current I_s, and DC voltage V_{dc}, as well as Fig. 3. Without a filter, the nature of the source current is identical to that of the load current. Fig. 4 and Fig. 5 show the MATLAB simulation results for steady state, dynamic condition of load, and parametric variation of the HSAPF system under sliding mode controller-2.

CONCLUSION

For HSAPF, a new robust controller design has been presented in this paper. Sliding mode controller2 establishes the control design by deriving the equivalent control law. This control law is extremely useful for generating switching patterns. The proposed controller's robustness was validated by analysing the performance of the power system under steady-state and transient conditions. The HSAPF's functionalities are improved with the use of this technique. SRF method is found to be the best for reference generation in the presence of switching losses and distortion in both source current and load voltage. Furthermore, the variable structure control method of the sliding mode controller-2 reduces tracking error distortion, suppresses chattering, and noise, and thus achieves perfect gain stability of the HSAPF system.

REFERENCES

1. Z. Zeng, H. Yang, S. Tang, and R. Zhao, “Objective-oriented power quality compensation of multifunctional grid-tied inverters and its application in microgrids,” *Power Electronics, IEEE Transactions on*, vol. 30, no. 3, pp. 1255–1265, 2015.
2. B. Nassif, W. Xu, and W. Freitas, “An investigation on the selection of filter topologies for passive filter applications,” *Power Delivery, IEEE Transactions on*, vol. 24, no. 3, pp. 1710–1718, 2009.
3. M. Ali, E. Laboure, and F. Costa, “Integrated active filter for differential-mode noise suppression,” *Power Electronics, IEEE Transactions on*, vol. 29, no. 3, pp. 1053–1057, 2014.
4. E. R. Ribeiro and I. Barbi, “Harmonic voltage reduction using a series active filter under different load conditions,” *Power Electronics, IEEE Transactions on*, vol. 21, no. 5, pp. 1394–1402, 2006.

5. G. Satyanarayana, K. Lakshmi Ganesh, Ch. Narendra Kumar and M. Vijaya Krishna, "A Critical Evaluation of Power Quality Features Using Hybrid Multi-Filter Conditioner Topology", *(IEEE Conference) International Conference on Green Computing, Communication and Conservation of Energy (ICGCE),* pp. 733-738, 2013.
6. Naga Raju Pechetti, Ch. Narendra Kumar and Ch. Rambabu, "DC Voltage Controller For High Power Statcom", *International Journal of Innovative Research & Studies (IJIRS), ISSN 2319-9725*,pp.428-443, Volume 3, Issue 1, January 2014.
7. B. Mallikarjuna Reddy, Ch. Narendra Kumar and Ch. Rambabu, "A ZVS-PWM Three Phase Current Fed Push Pull DC-DC Converter With Fuel Cell Input", *International Journal Of Science, Engineering And Technology Research (IJSETR), ISSN: 2278 – 7798*, Volume 3, Issue 12, December 2014.
8. Kambham Ravi Shankar, Ch. Narendra Kumar and Ch. Rambabu, "Power Quality Improvement by UPQC based on Voltage Source Converters", *International Journal of Research in Science & Technology (IJRST), ISSN: 2349-0845,* Volume 2, Issue 1, January 2015.
9. China Subba Rao Mamidi, Ch. Narendra Kumar and Ch. Rambabu, "ZVS Inverter System for an Induction Motor drive with Interconnection of PV Cell", *International Journal of Engineering Associates (IJEA), ISSN: 2320-0804,*Volume 4, Issue 3, pp.30-36, March 2015.
10. Shaik Mahaboob Subhani, Ch. Narendra Kumar and Ch. Rambabu, "Bidirectional Resonant DC-DC Converter using Fuzzy Logic Controller for Hybrid Power Generators", *International Journal of Advanced Technology and Innovative Research (IJATIR), ISSN: 2348-2370,*Volume 8, Issue 3, pp. 520-528, March 2016.
11. F. Z. Peng, H. Akagi, and A. Nabae, "A new approach to harmonic compensation in power systems-a combined system of shunt passive and series active filters," *Industry Applications, IEEE Transactions on*, vol. 26, no. 6, pp. 983–990, 1990.
12. S. Diptimayee Swain, P. K. Ray, and K. Mohanty, "Voltage compensation and stability analysis of hybrid series active filter for harmonic components," in *India Conference (INDICON), 2013 Annual IEEE*, pp. 1–6, IEEE, 2013.
13. W. Tangtheerajaroonwong, T. Hatada, K. Wada, and H. Akagi, "Design and performance of a transformerless shunt hybrid filter integrated into a three-phase diode rectifier," *Power Electronics, IEEE Transactions on*, vol. 22, no. 5, pp. 1882–1889, 2007.
14. W. Guo, J. Wu, and D. Xu, "A novel sliding mode control of a high-voltage transformerless hybrid shunt active power filter," in *Industrial Electronics and Applications, 2009. ICIEA 2009. 4th IEEE Conference on*, pp. 2908–2913, IEEE, 2009.
15. B. Kedjar and K. Al-Haddad, "Dsp-based implementation of an lqr with integral action for a three-phase three-wire shunt active power filter," *Industrial Electronics, IEEE Transactions on*, vol. 56, no. 8, pp. 2821–2828, 2009.
16. R. Panigrahi, B. Subudhi, and P. C. Panda, "A robust lqg servo control strategy of shunt-active power filter for power quality enhancement," *Power Electronics, IEEE Transactions on*, vol. 31, no. 4, pp. 2860–2869, 2016.
17. L. M. Fridman, "Singularly perturbed analysis of chattering in relay control systems," *IEEE Transactions on Automatic Control*, vol. 47, no. 12, pp. 2079–2084, 2002.
18. M. A. Mulla, R. Chudamani, and A. Chowdhury, "A novel control method for series hybrid active power filter working under unbalanced supply conditions," *International Journal of Electrical Power & Energy Systems*, vol. 64, pp. 328–339, 2015.
19. S. Rahmani, K. Al-Haddad, and H. Y. Kanaan, "Average model- ing and hybrid control of a three-phase series hybrid power filter," in *Industrial Electronics, 2006 IEEE International Symposium on*, vol. 2, pp. 919–924, IEEE, 2006.
20. S. D. Swain and P. K. Ray, "Harmonic current and voltage compensation using hsapf based on hybrid control approach for synchronous reference frame method," *International Journal of Electrical Power & Energy Systems*, vol. 75, pp. 83–90, 2016.
21. H. De Battista and R. J. Mantz, "Harmonic series compensators in power systems: their control via sliding mode," *Control Systems Technology, IEEE Transactions on*, vol. 8, no. 6, pp. 939–947, 2000.

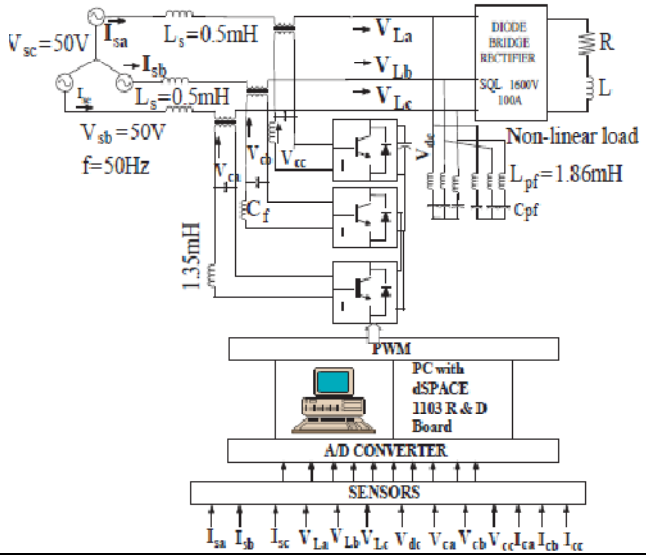

Fig. 1 Schematic diagram of a Hybrid Series Active Power Filter (HSAPF)

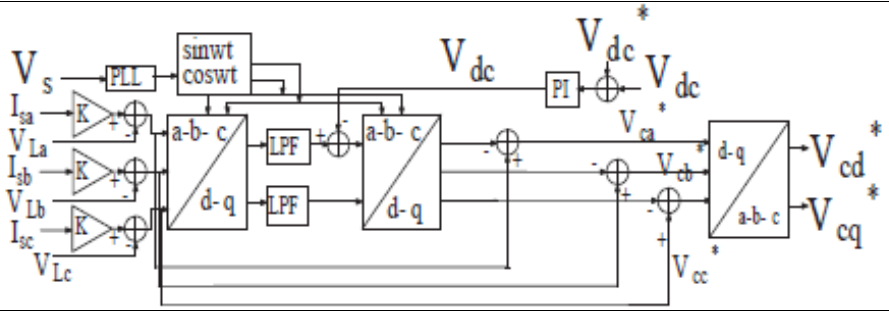

Fig. 2 Reference Generation Scheme

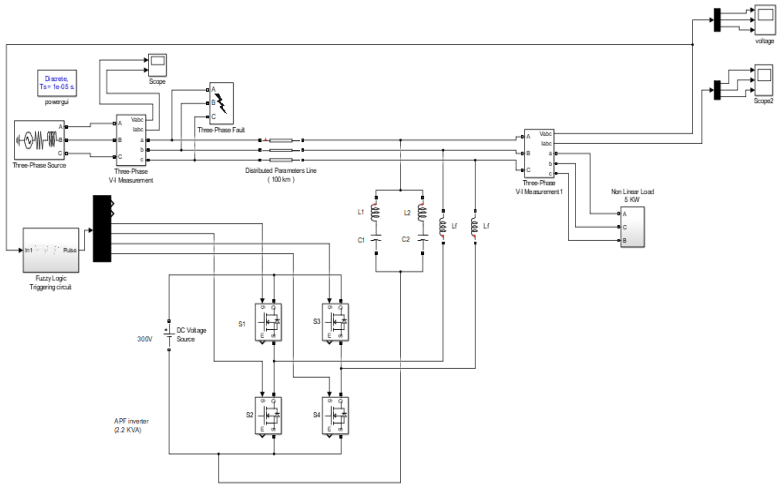

Fig.3 Simulation Diagram

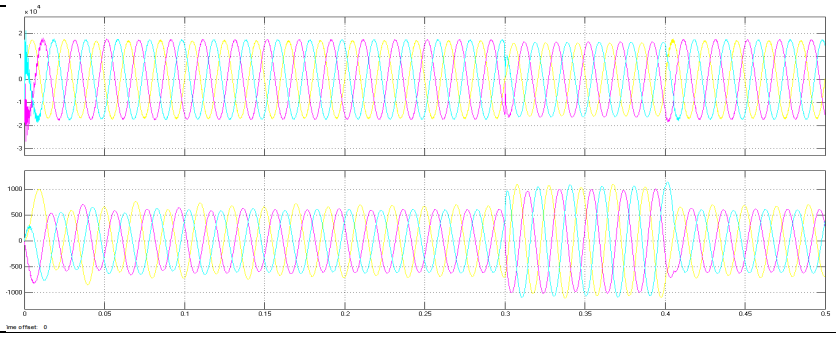

Fig. 4 Simulation Waveforms (Before Fault)

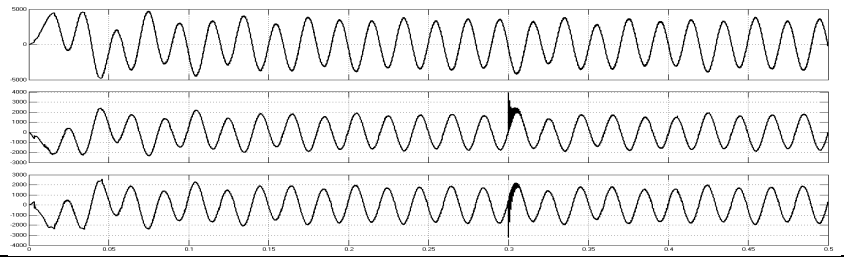

Fig. 5 Simulation Waveforms (After Fault)

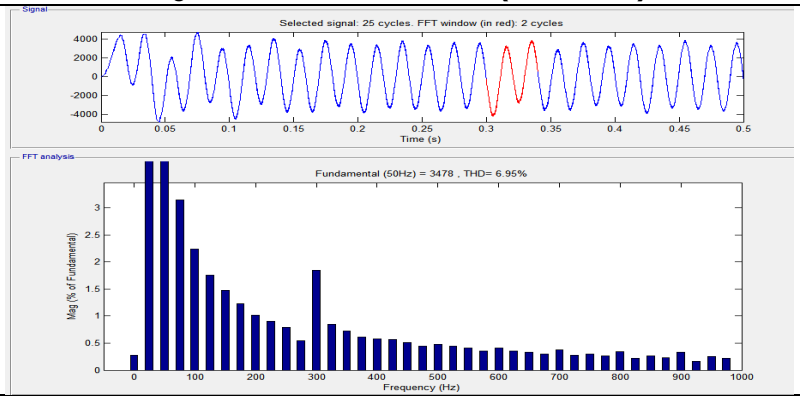

Fig. 6 THD Analysis (Before Fault)

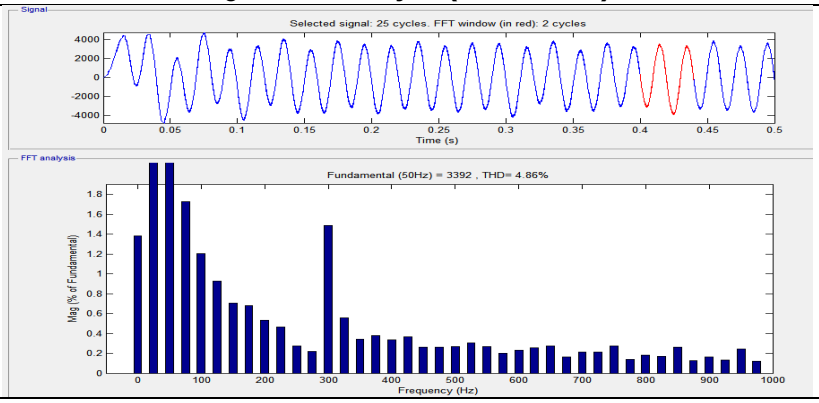

Fig. 7 THD Analysis (After Fault)

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RESEARCH ARTICLE

Design of Multilevel Inverter with Two DC Sources

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ABSTRACT

Paper presented one 13-level diode clipped staggered inverter (k-type) with dc sources. The planned staggered is planned dependent on two consecutive T-Type modules for certain changes up them. The component is K Type. The setup of K-type gives two additional direct current joins in capacitors to accomplish extra level toward make the waveform. The component requirements lesser segments together with two Direct current sources, two capacitors, 14 semiconductors. It very well may be utilized in power applications with various DC sources). It tends to be effortlessly planned in two methodologies in course plans to shape high voltage yields with low weight on semiconductors and bringing down the quantity of gadgets. This capacity can be utilized in some extraordinary applications, for example, sun powered ranch alongside a ton of DC sources. DC sources can be special voltage amplitudes. The ordinary techniques, it very well may be viewed as one inverter for every DC assets and fix the yield voltage a similar sufficiency. It expands intricacy and misfortunes from this perspective, however in diode clasped staggered inverters, it is feasible to consolidate some DC assets together and produce a one of a kind AC yield. It decreases the quantity of isolated inverter, misfortunes segments and so on Recreations are execute in MATLAB/Simulink and a model is carried out in the force hardware research facility which the reproduction.

Keywords: asymmetric, staggered inverter, power gadgets, capacitors, self-charging, level control exchanging.

INTRODUCTION

Multilevel inverters (MLIs) contain giving the solid and great voltage source converters to combine the direct current power frameworks to the alternative power frameworks. MLIs with various course of action are one of the fascinating gadgets with regards to control hardware. The nature of intend spreads them like a reach application in power framework. The capacities of MLIs in medium/high force application against two levels inverters compose them driving converter inside photovoltaic frameworks [1], HVDC used for transmission line, wind turbine [2], dynamic force filer drives frameworks, electrical vehicle and force lattice, Multilevel inverter contain elevated goal on the yield voltage and low symphonious parts due to a high number of yield level. They include little weight going on switches, particularity because of course association capacity. MLIs are brought into Flying Capacitor (FC) Neutral Point Clamped (NPC), Cascade H-connect (CHB) [3]. Lopsided DC connections and high weight on switches are the drawbacks of Neutral Point Clamped and Flying Capacitor which are including tremendous capacitors. In this way, CHB types are engaged by decreased numbers and scientists, of parts are focused on in the setup of CHB topologies [4]. This sort of geographies are equivalent from various kinds of viewpoints like the quantity of levels, the quantity of semiconductors, the quantity of DC sources, complete standing voltage the inborn making of negative levels and so on some surveying reads are introduced in for last decade topologies[5]. A direct current basis produced one of the level with two switches inside and make one module mutually. The module was associated in series with to make parcel of levels. All levels are positive and it needs an extra circuit to make negative levels. H-connect is extra to the series modules in for flight of stairs sinusoidal waveform. The semiconductors in H-connect circuits which make negative voltage levels endure far above the ground exchanging pressure. By and large, staggered inverters orchestrate various associations of semiconductor changes to combine a few low voltage steps to frame a yield waveform [6]. Utilizing lower parts to deliver more noteworthy yield voltage levels are one of the significant issues of multilevel inverters. Staggered inverters with inconsistent DC joins present another sort of design which diminished the quantity of parts alongside more noteworthy yield voltage levels. Modules are planned dependent on adding or taking away of DC joins by power hardware semiconductors [6]. A different side, the weight on switches can be there thinking about in topsy-turvy staggered inverters because of various DC interface. The weight on switches is presented with complete standing voltage which is an absolute high voltage of each switch off mode [7]. Present intersection switches for inverse extremity of DC source to produce undeniable levels and isolating of weight on switches. H-connect with various degree of DC joins is introduced during more elevated levels in this geographies are alongside weight on switches needs higher rate semiconductor [8]. Cross breed type geographies are proposed as another sort of staggered inverters in presented modules with low semiconductors with intrinsic negative levels dependent on the accomplishing of questionable levels from four direct current sources [9,10].

Existing System

Multilevel converters (MLI) are brought into Neutral Point Clamped Flying Capacitor Cascade H-connect. Unequal DC connections and elevated weight going on switches be the hindrances of NPC and FC which are including enormous capacitors. Consequently, CHB types are engaged by analysts, and diminished quantities of parts are designated in the setup of CHB geographies.

- Extended H-connect with various measures of DC joins is introduced. More elevated levels in these geographies are alongside weight going on switches that needs elevated rate semiconductor
- Hybrid type geographies are proposed as another kind of MLIs modules with semiconductors with inborn negative levels dependent of complishing of greatest levels from four DC sources.
- More yield voltage levels can be accomplished with a similar DC sources. Upgraded the design of capacitors near eliminate some DC sources. Utilize a solitary source to create yield levels, albeit the quantity of semiconductors has been expanded and charging/releasing of capacitors and switches driving become confounded. Some different geographies are proposed with blended DC sources and capacitors as the step measured designs, despite the fact that H-connect are useful in the circuits.

Proposed Module

This paper gives a course of action of semiconductor blended DC sources and capacitors as direct current connects toward accomplish most extreme voltage levels as of DC sources which further develop monetary execution cost and force superiority. This module utilizes only two inconsistent DC sources with the measure of 1VDC, 2VDC to produce 13 yield voltage levels. Then again, a halter kilter staggered module is acquainted with produces 6 +ve levels, 6 -ve levels and nothing level (absolutely 13 levels) with no added circuit to make negative voltage levels. 14 force gadgets switches and two capacitors are executed in the proposed module. The module can be associated in series as course association effectively to deliver more and higher yield voltage levels. Fig.1 shows an overall calculated chart of staggered inverters. An appropriate planning of force converter can accomplish greatest yield levels from two DC sources. It is feasible to utilize capacitors to make some additional DC connects to get a larger number of levels than the assumption. In this sort of arrangement, the charge way of capacitors ought to be given notwithstanding the yield levels ways. It is fascinating to don't utilizing an extra circuit of charging of capacitors. At that point a savvy planning for the staggered inverter is introduced as follow:

Module Configuration

There be two DC sources by various sums as 1VDC and 2VDC. Utilizing inconsistent DC hotspots for uneven staggered inverters items diverse amount of yield voltage levels in less semiconductors and low consonant parts too. It very well may be smarter to make two additional DC joins with capacitors. It gives four DC joins, completely. Fig.2 presents the proposed module with another part game plan including 14 switches (8 unidirectional switches and 3 bidirectional switches), 14 diodes and 2 inconsistent DC source and 2 capacitors. This design creates six positive levels, six negative levels and zero level (13 levels completely). The state of proposed geography is like Kite and it is named "K-Type" (Kite Type). The fundamental idea of this circuit is making various ways from various sides of a DC connect to be associated with other DC connects to create negative levels to eliminate H-connect. It is recognizable that DC source with 1VDC accuses capacitor of 1VDC, and DC source with 2VDC accuses the capacitor of 2VDC with no extra circuit. Fig.2 and Fig 3.The Mat lab circuit diagram of open loop k type inverter. The planning of the module and their exchanging ways are chosen sagaciously so that There are no sure post of DC joins on the anode side of diode to lead. Likewise, Fig4: The wave form Output current and voltage waveform of open loop K-type inverter.In this manner, diodes extremity and bidirectional switches ensure for stifling of switches that short circuiting will be not happened in the module. Fig5: The wave form Output current and voltage waveform of closed loop K-type inverter and Fig6: The wave form Output current and voltage waveform of closed loop K-type inverter . Fig 7.The Mat lab circuit diagram of closed loop control circuit and Fig 8:The output waveform of 13-level Multilevel inverter**.**

CONCLUSION

Paper presents another reconfiguration module for topsy-turvy staggered inverters within which is capacitors are utilized as direct current connects to make the levels for flight of stairs waveforms. This arrangement of staggered converter makes a decrease in DC sources. Then again, the feasible to produce 13 levels with lower DC sources. The proposed module of staggered inverter creates 13 levels of two inconsistent DC sources (2VDC and 1VDC). It likewise includes two on expenses capacitors and 14 semiconductor switches. The capacitor is self-charging with no additional circuit. The lower number of segments makes it attractive to use in large scope of utilizations. The module is schematized as two consecutive T-type inverters and different changes up it. Likewise, it tends to be there associated with courses particular which lead to a secluded geography with additional voltage levels at higher voltages. The proposed module makes the inborn formation of the negative voltage levels with no extra circuit, (for example, H-connect circuit). Closest level control exchanging tweak (NLC) conspire is applied to accomplish great sinusoidal yield voltage.

REFERENCES

1. Essakiappan, S.; Krishnamoorthy, H.S.; Enjeti, P.; Balog, R.S.; Ahmed, S., "Multilevel Medium-Frequency Link Inverter for Utility Scale Photovoltaic Integration," in Power Electronics, IEEE Transactions on , vol.30, no.7, pp.3674-3684, July 2015
2. A. Nami, J. Liang, F. Dijkhuizen and G. D. Demetriades, "Modular Multilevel Converters for HVDC Applications: Review on Converter Cells and Functionalities," in *IEEE Transactions on Power Electronics*, vol. 30, no. 1, pp.18-36, Jan. 2015.
3. I. A. Gowaid, G. P. Adam, A. M. Massoud, S. Ahmed and B. W. Williams, "Hybrid and Modular Multilevel Converter Designs for Isolated HVDC–DC Converters," in IEEE Journal of Emerging and Selected Topics in Power Electronics, vol. 6, no.1, pp. 188-202, March 2018.
4. Xibo Yuan, "A Set of Multilevel Modular Medium-Voltage High Power Converters for 10-MW Wind Turbines," in Sustainable Energy, IEEE Transactionson , vol.5, no.2, pp.524-534, April 2014
5. Gupta, K.K.; Ranjan, A.; Bhatnagar, P.; Sahu, L.K.; Jain, S., "Multilevel Inverter Topologies With Reduced Device Count: A Review," in Power Electronics, IEEE Transactions on , vol.31, no.1, pp.135-151, Jan. 2016
6. S. Debnath, J. Qin, B. Bahrani, M. Saeedifard and P. Barbosa, "Operation, Control, and Applications of the Modular Multilevel Converter: A Review," in *IEEE Transactions on Power Electronics*, vol. 30, no. 1, pp. 37-53, Jan. 2015.
7. J. Venkataramanaiah, Y. Suresh, Anup Kumar Panda," A review on symmetric, asymmetric, hybrid and single DC sources based multilevel inverter topologies," Renewable and Sustainable Energy Reviews, Volume 76, Pages 788-812, 2017
8. E. Babaei and S. H. Hosseini, "New cascaded multilevel inverter topology with minimum number of switches," Elsevier J. Energy Convers. Manage, vol. 50, no. 11, pp. 2761–2767, Nov. 2009.
9. Babaei, E. Kangarlu, M.F. ; Sabahi, M. "Extended multilevel converters: an attempt to reduce the number of independent DC voltage sources in cascaded multilevel converters" IET Power Electronics, Volume 7, Issue 1, January 2014, p. 157 – 166
10. K.K. Gupta and S. Jain, "Topology for multilevel inverters to attain maximum number of levels from given DC sources" IET Power Electronics, Volume 5, Issue 4, April 2012, p. 435 – 446.

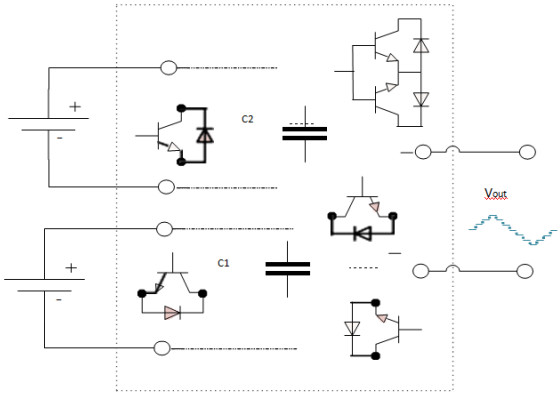

Fig 1: The general conceptual asymmetric MLIs with capacitors

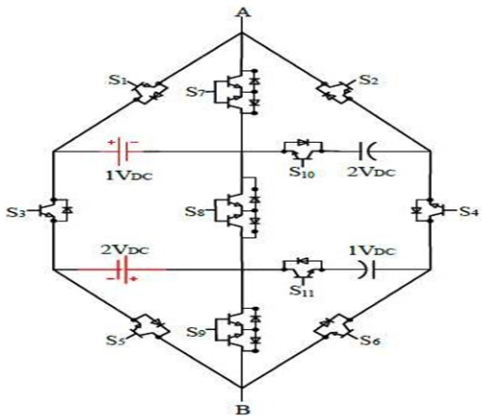

Fig 2: The proposed module of multilevel inverter

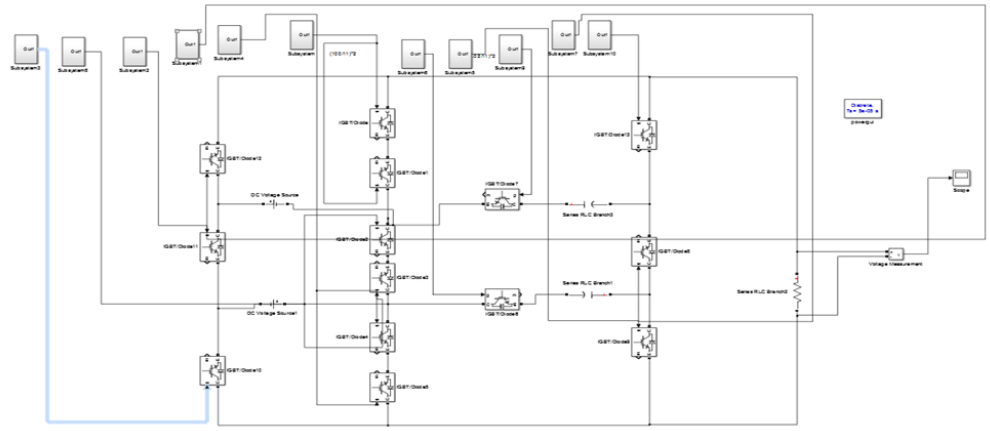

Fig 3: The Mat lab circuit diagram of open loop k type inverter

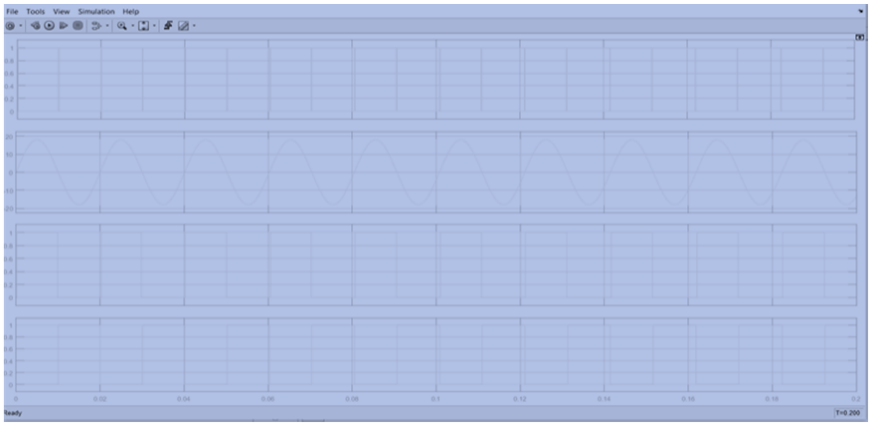

Fig 4: The wave form Output current and voltage waveform of open loop K-type inverter

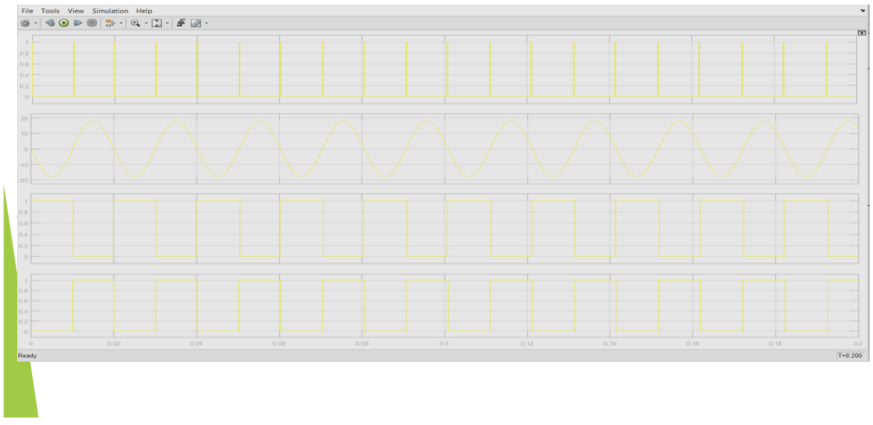

Fig 5: The Mat lab circuit diagram of closed loop k type inverter

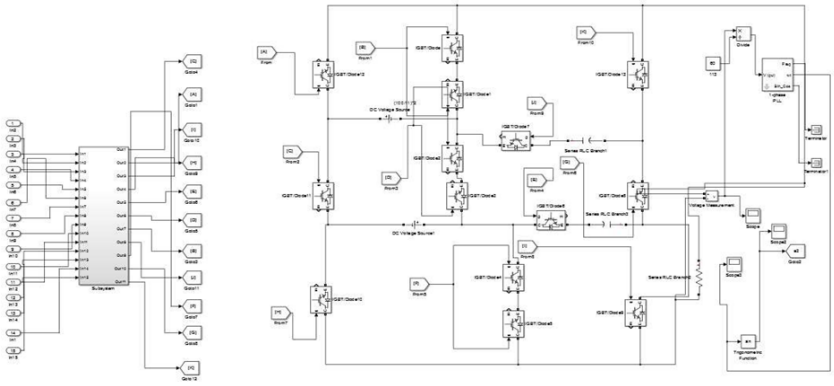

Fig 6: The wave form Output current and voltage waveform of closed loop K-type inverter

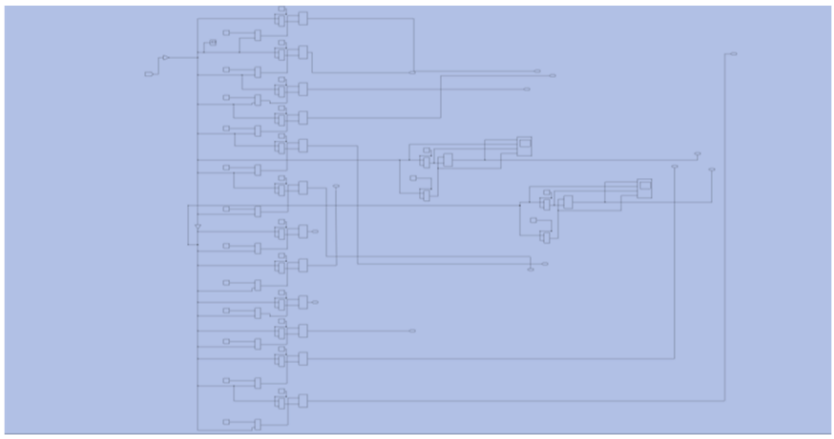

Fig 7: The Mat lab circuit diagram of closed loop control circuit

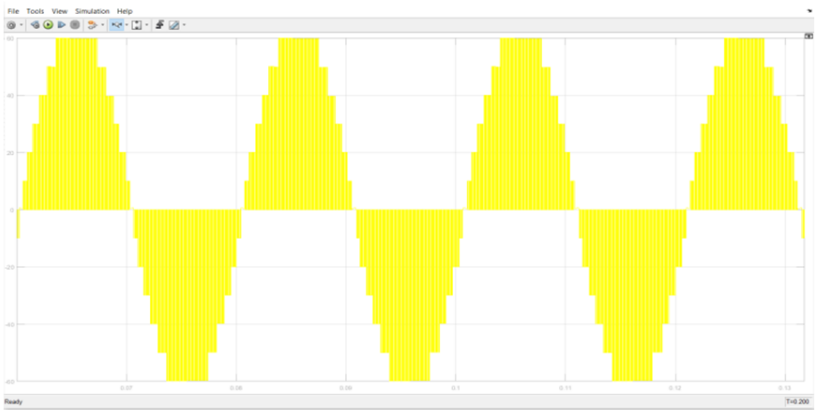

Fig 8: The output waveform of 13-level Multilevel inverter

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REVIEW ARTICLE

Clinical Review on Breast Cancer-An Update

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ABSTRACT

Breast cancer is the second biggest cause of cancer death in women after lung cancer. It is estimated that around one in every eight women will develop breast cancer during her lifetime. Since the previous two decades, research into breast cancer has resulted in remarkable advancements in our understanding of the illness, resulting in more effective and less hazardous treatments for women with breast cancer. Public education and increased screening have led to early detection of cancers at stages that are amenable to complete surgical resection and curative treatments. As a result, breast cancer survival rates have increased dramatically in recent years, particularly among younger women. This review discusses history, epidemiology, different forms of breast cancer, their causes, clinical symptoms, and the various non-drug (such as surgery and radiation) and drug-based (such as chemotherapy, gene therapy, and so on) approaches to treating them.

Keywords: Breast cancer, History, Epidemiology, Chemotherapy.

INTRODUCTION

Cancer has the potential to be a major cause of illness and mortality in both developing and industrialized countries, such as Asian countries, America, Africa, and Australia, among others. Women are most commonly affected by breast cancer, which is defined by an uncontrollable proliferation of abnormal cells within the milk production glands of the breast. Breast cancer is the most prevalent type of cancer in women. It is the most common type of cancer in females, and it develops most frequently in postmenopausal women over the age of fifty, as well as in young women. It is possible for men to develop breast carcinoma as well, although it is extremely rare, accounting for less than 1% of all cancer cases. Breast cancer in its early stage is a non-invasive illness that is contained inside the ducts or lobules of the breast and has not migrated into the surrounding healthy tissue. Invasive breast cancer has

spread beyond the ducts and into healthy breast tissue before the ducts. A tumor that originates in the breast tissue of both men and women [1] is known as breast cancer. Ductal carcinoma is the most common type of breast cancer, accounting for the majority of cases [2]. A few number of cells begin to grow within the sacs or lobules (lobular cancers). Carcinoma has the potential to spread to the lymph glands as well as to other regions of the body, such as the bones and liver. Carcinoma is often discovered during a screening check, before any symptoms have manifested themselves, or after a young woman feels a lump in her breast. Early 1980s, screening mammography has resulted in significant improvements in the early identification of breast cancer [3].Mammography is a type of specialist medical imaging that scans the breasts with a low-dose x-ray system using a low dosage of radiation. A mammography exam, often known as a mammogram, is used to aid in the early detection and diagnosis of breast disorders in female patients. Screening mammography are performed to detect cancer in the breasts of women who do not exhibit any symptoms [4]. The male breast tissue is similar to that of the female in that it is composed of fibrous tissues, fine ducts, fat, and glandular elements or lobules, just like the female. Breast cancer can affect both men and women, however male breast cancer is extremely rare around the world [5].

History of Breast Cancer

More than 3,500 years ago, the ancient Egyptians became the first people to become aware of the condition. Hippocrates provided an explanation of breast cancer. In addition, he argued that the human body is composed of four senses of humour – phlegm, blood, black bile, and yellow bile – that are responsible for a variety of bodily functions. Hippocrates believed that an excess of black bile was responsible for the development of cancer. Galen goes into great detail on the cancer after that, in the year 200 A.D. He prescribed remedies such as castor oil, opium, sulfur, licorice, salves, and other natural products. It is the only treatment that is curative for mammary tumors. The French physician Francois de la Boe Sylvius, who lived around 1680, believed that cancer did not originate from the dark bile. Using a chemical function that moves lymphatic fluids from acidic to acrid as an example [5,] he argued that it was the cause [6]. In the 1730s, Claude-Deshais Gendron, a Parisian physician, also disputed Galen's systemic theory, claiming that cancer arose when nerve and glandular tissue became entangled with lymph veins and bled. In 1713, Bernardino Ramazzini formulated the hypothesis that women are more likely than men to get mamilla cancer as a result of a lack of sexual activity. Ramazzini stated that irregular sexual activity, reproductive organs, and the mammary gland could be disbanded or destroyed, which could lead to the development of malignancies. Another researcher, Friedrich Hoffman of Prussia, hypothesized that females who have proper sex but still had tumors that have matured were engaging in vigorous intercourse with their partners. Because of lymphatic obstruction, these symptoms are frequently present. A great deal of study has since been done on breast cancer and its treatments by a large number of scientists. Following extensive investigation, experts hypothesized that surgical removal of the tumor could aid in the treatment of breast cancer. By the mid-nineteenth century, surgery was the only treatment option for women with breast cancer. The development of antiseptics, anaesthesia, and blood transfusion techniques during this period also increased the likelihood of surviving a surgical procedure [6].Fisher published his findings in 1976, employing a simpler breast-conserving operation followed by radiation or chemotherapy as a treatment option. He understood that these were as effective as a radical mastectomy in some cases. In line with the improvement of current medicine, by 1995, less than 10% of Mamilla cancer-affected women had undergone a mastectomy. Additionally, this generation witnessed the emergence of novel treatments for breast cancer, including surgical procedures, biological treatments, and hormonal therapy [7].Mammography was also created to aid in the early detection of malignancies in the breast. The genes that cause breast tumors were then identified and classified as follows: BRCA 1, BRCA 2, ATM.

Epidemiology

It is estimated that breast cancer accounts for 10.4 percent of all malignancies worldwide, making it the most frequent malignant tumour in the globe. It is the leading cause of death among women between the ages of 20 and 50 years [8-10]. Breast cancer incidence has grown globally since 1980, when 641,000 cases were reported. It is reasonable to estimate that 1.67 million new cancer cases were detected worldwide in 2012, according to a WHO survey conducted in an Asian country. From the year 2015 to the present, the American and South American

populations have been affected by 0.3 percent to 0.4 percent per year, while black and American Indian populations have been affected by 0.7 percent to 0.8 percent per year, and Asian women have been affected by 1.8 percent per year [11-14]. According to the latest statistics, the death rate from breast cancer has decreased by 40% in 2016. Year Australia, roughly 730 new cases of cancer will be diagnosed in 2017. In the United States, it was predicted that 40,920 women would die from breast cancer in the year 2018. In the United States, more than 1.7 million new tumor cases are expected to be detected in the year 2019. [15-16] Breast cancer affects 268,600 women and 670 men in the United States; of these, 41,760 women and 500 men are in danger of developing the disease. Breast cancer claimed the lives of 94 women in Africa [17]. It is estimated that more than half of the population in many low- and moderate-income nations, including India, does not have access to a properly organized and well-regulated cancer care system. Women between the ages of 15 and 49 years old are twice as likely as men to get carcinoma in developing nations as they are in affluent countries [18-19].

Etiology

A past history of breast cancer: The risk of breast breast cancer in the other breast increases in women who have had breast cancer in one of her breasts [19, 20].

Family history: patients may be at increased risk of having breast cancer if multiple members of their family have had specific cancers [21].

Genetic causes: Family history of breast cancer has long been recognized as a risk factor for the disease. The importance of maternal and paternal relatives cannot be overstated. A close relative who has developed breast cancer at a young age, has cancer in both breasts, or has cancer in both breasts has the highest risk of developing the disease. First-degree relatives (mother, sister, and daughter) are the most essential when it comes to determining risk. Another factor to consider is the presence of several second-degree relatives (grandmother, aunt) who have been diagnosed with breast cancer. When a male develops breast cancer, he increases the risk for all of his close female relatives. Inheriting faulty genes such as BRCA1 and BRCA2 significantly increases the chance of breast cancer, with a lifetime risk of between 40 and 8S percent thought to be associated with the genes. Women who carry the BRCA1 gene are more likely than the general population to acquire breast cancer at an early age [22].

Hormone: Breast cancer may be triggered by a change in the level of hormone in the blood. It can be accompanied with the onset and cessation of periods (Menstrual Cycle), pregnancy at a young age, hormone replacement therapy, the use of oral pills, and other symptoms [23].

Life style and dietary: Breast cancer is thought to be caused by a sedentary lifestyle, a high dietary intake of fat, and obesity, particularly in postmenopausal women. The consumption of alcoholic beverages is also a contributing factor to breast cancer. The danger grows in direct proportion to the amount of alcohol taken. Women who drink between two and five alcoholic beverages per day have a risk of developing breast cancer that is approximately one and a half times higher than that of non-drinkers [24].

Environment: It is known that women who deal with low doses of radiation over a long period of time, such as X-ray technicians, are at a modest increased risk of cancer [25].

Symptoms of Breast Cancer

A new lump or mass within the breast that you can feel could be the first sign of breast carcinoma. The lump is painless, hard, and has uneven edges, all of which indicate that it is cancer. Cancers, on the other hand, are often delicate, soft, and rounded. The following symptoms will be present throughout the course of breast cancer such as Swelling of the entire breast or a portion of the breast, dimpling, breast discomfort, the inward curving of the breast, breast redness, thickening of the breast tissue, or skin on the breast and discharge from the breasts that is not breast milk. In some cases, a mass might be found in the underarm space [26,27].

Risk factors for mammary cancer include: Many investigations have discovered that tumors are the cause of a wide range of disorders in women. The following are the most significant risk factors for developing breast carcinoma:

Growing older- The risk of breast carcinoma increases with age, with the majority of breast tumors being diagnosed beyond the age of fifty. Genetic mutations- Genetic changes include changes in the genes BRCA1 and BRCA2, which are breast cancer susceptibility genes. Women who are born with these genetic alterations are at an increased risk of developing breast and ovarian cancer [28]. Early catamenial period- Women who start their periods before the age of twelve are exposed to hormones for a longer period of time, increasing their risk of developing breast cancer by a little margin.If the main germination occurs just once, at the age of thirty, and the terminal fertilization does not occur, the risk of breast carcinoma is low. Late or no physiological state. Late biological time - If you were born after the age of 55, you have a higher risk of developing breast cancer than women who were born earlier in life [29]. Obesity is associated with a higher risk of developing breast cancer in older women than in those of normal weight.
Using combined secretion therapy- Taking hormones to replace lost progestogen and oestrogen in biological life for more than 5 years increases the risk of developing breast carcinoma. When the hormones progestogen and oestrogen are combined, the likelihood of developing breast cancer increases significantly. Contraceptive pill or oral contraceptive pill -There are several different types of oral contraceptives. Pills have been demonstrated to increase the risk of developing cancer [30].

Types of Breast Cancer

All types of breast cancer begin in the breast tissue, which is made up of lobules, which are milk-producing glands, and milk-ducts, which connect the lobules to the teat. Breast carcinoma is the most rapidly increasing malignancy in women today, both in industrialized and developing nations [31].
Breast cancer can be divided into two categories: Non-invasive and Invasive.

Non-invasive: Ductal carcinoma in situ (DCIS) could be a disorder in which abnormal cells replace the normal epithelial cells of the breast ducts, causing the ducts and lobules to become significantly larger than they were originally. As a result, DCIS is considered a non-invasive type of cancer since the aberrant cells have not progressed beyond the layer of cells from whence they emerged. It is the most frequent type of in situ breast cancer, accounting for around 83 percent of all in situ cases diagnosed between 2008 and 2012 in the United States. DCIS may or may not progress to invasive cancer; nevertheless, a lot of these tumours grow at such a sluggish rate that they may not pose a threat to a woman's health even if left untreated [32].

Invasive: This is a term that most people are familiar with; the majority of breast cancers are invasive, or infiltrating. These tumors spread through the surrounding breast tissue after breaking through the walls of the glands or ducts from which they originated. Various parts of the breast, including the canals, lobules, and, in a few cases, the spaces between the tissues [33], can be affected by breast cancer. Papillary carcinoma, lobular carcinoma, Apocrine carcinoma, Inflammatory carcinoma, Repetitive carcinoma, and pathologic process breast carcinoma are all types of invasive breast cancer [34]. Invasive ductal carcinoma is the most common type of invasive breast carcinoma, followed by lobular carcinoma and papillary carcinoma.

Breast Cancer Stages

Breast cancer stages range from 0 to 4, with the first stage being curable breast carcinoma and the last stage being non-curable (metastatic) breast carcinoma.
Breast cancer is classified into four stages: 0-4
Stage 0: At this stage, the tumour is quite small in comparison to the rest of the body. Despite the fact that tumour cells have expanded to the breast area, they are unable to move to other tissues or organs in the surrounding area.

Stage I: This is the earliest stage of breast cancer development, and it indicates that the tumour is less than 2 cm in size. A total of two stages are included in stage I.

Stage IA: At this stage, the tumour is only a small size. Breast carcinomas that are up to 2cm in size and have not spread outside of the breast, as well as no lymph nodes, are considered benign.
Stage IB: The tumour has migrated to the lymph nodes and has grown to a size greater than 2cm [35].

Stage II: At this stage, the tumour has developed and spread to nearby tissues. Tumours can range in size from 2 to 5 cm. Stage II is divided into two groups, which are referred to as IIA and IIB.
During **Stage IIA,** the tumour has not progressed farther into the breast. If the size of the carcinoma is less than 2 cm, the cancer has the potential to expand to the axillary nodes.
Stage IIB: The tumour has grown to a significant size at this point. If the tumour is less than 2 cm in diameter and bigger than 5 cm in diameter, it has the potential to spread to 1-3 axillary lymph nodes. Even if the tumour is larger than 5 cm in diameter, it does not spread to the axillary lymph nodes.

Stage III: At this stage, the tumour has not migrated to the bones or organs of the body. The tumour has grown to a significant size at this point. Tumours can grow to be up to 5 cm in diameter or greater. Stage III is divided into three stages, which are denoted by the letters IIIA, IIIB, and IIIC.
Stage IIIA: During this stage, the tumour grows in a chain-like pattern from the underarm to the collar bone. The lymph node has been significantly expanded.
Stage IIIB: During this stage, the tumour begins to expand within the chest cavity and the surrounding skin. Tumours can be of any shape or size. At this stage, the tumour has not yet migrated to the lymph nodes, but it has spread to the chest wall and is causing discomfort.
Having cancer in ten or more lymph nodes indicates that it has spread above or below the collarbone, according to **stage IIIC.** In IIIC, fewer lymph nodes are afflicted outside the breast, but those that are affected inside the breast are swollen or malignant.

Stage IV: The tumour might be of any size at this point in the process. The tumour has spread throughout the body, including the liver, brain, lungs, and bones. Breast cancer cells have spread to the lymph nodes and breast tissue. The metastatic stage is the point at which cancer has spread [36].

Breast Cancer Therapy

Treatment options for breast cancer vary based on the stage of the disease. There are a variety of approaches of treating carcinoma. The following are breast cancer treatments: Surgery, Radiation therapy, Chemotherapy, Secretion therapy and Specific medical treatment.

Surgery: For many individuals, surgery is the most effective therapy option. Surgery is the term used to describe the removal of a tumour during an operation. This therapy is also utilized to examine the lymph nodes in the axilla that are close. It consists of two types, which are lumpectomy and mastectomy. Lumpectomy surgery is the excision of a tumour using a surgical procedure known as a lumpectomy. Breast conserving surgery and quadrantectomy are two more names for this procedure. In this case, two genes, BRCA1 and BRCA2, are involved in the gene mutation process. Women who have been newly diagnosed with breast cancer and have BRCA1 or BRCA2 gene mutations may be eligible for breast conserving surgery if they have these mutations. Mastectomy: This procedure involves the removal of the entire breast, rather than only the lymph nodes in the body [37].The skin can be preserved with skin sparing mastectomy, and the nipple can be preserved through nipple sparing mastectomy while using this treatment method.

Radiotherapy: In this treatment, high-energy radiation (such as x-rays) are utilized to eliminate cancer cells during the course of the treatment. Radiation sources are classified into two categories: external beam radiation and internal beam radiation. External beam emission (EBE) radiation therapy is the most common method of radiation therapy. Emission is visible from the outside of a vehicle's body. When using this technology, a radioactive substance is delivered to a specific spot within the body, and the emission process is extended over an extended length of time.

Chemotherapy: Chemotherapy is used prior to surgery (neo-adjuvant treatment).The goal is to reduce the size of the tumour and also to prepare for more extensive surgery. When surgery is performed in order to limit the likelihood of cancer cells entering our bodies. Chemotherapy is a treatment option for breast cancer that is available in numerous forms. Docetaxel, Paclitaxel, Doxorubicin, Epirubicin, Capecitabine, Transtuzumab, and Carboplatin are some of the most often used chemotherapy medicines.

Secretion therapy: It is used to treat a variety of conditions. This treatment is typically based on the use of medications that either block or impede the activity of the sex hormones oestrogen and progesterone. It can be found in nearly every part of the body and is not restricted to the breasts alone.

Targeted therapy: Precision medicine is a relatively recent approach to cancer treatment, and it is used to target specific biological processes that are usually necessary for tumour growth to be successful. Targeted therapy can include the use of antibodies, vaccinations, and sequencing therapies, among other approaches. These medicines are effective against all cells that are expanding fast throughout the body [38,39].

Gene Therapy: The current understanding of the roles played by proto-oncogenes and tumour suppressor genes in the development of malignancy has prompted the creation of gene therapy strategies aimed at either ablating or restoring these genes, as appropriate. Alternatively, cancer cells are equipped with the potential to convert a systemically delivered prodrug into a poisonous metabolite, or a target for destruction by replicating viral vectors, in order to maximize their chances of survival. The transfer of drug resistance genes into normal cells, on the other hand, has the potential to give chemo-protection during high-dose antineoplastic treatment. Finally, immune system modification has the potential to activate anticancer drug defense mechanisms **[**40].

CONCLUSION

This review explains about the breast carcinoma, which helps to reduce the risk factor and control the death rate. Due to the hypersecretion of sexual hormones like (progesterone and estrogens) and blockage of ducts is the major reason to cause breastcancer. So, excess intake of pills that contain the high quantity of steroid hormone and progestin so it should be avoided. Suppose it will take regularly as a result will be increase the chance of developing cancer. And also weight should be maintained because overweight ladies have a better risk of obtaining carcinoma than those who have traditional weight.

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REFERENCES

1. Greenall MJ, Morris PJ, Malt RA. Benign conditions of the breast. Oxford text book of surgery. New York: Oxford Medical Publication. 1994:789-808.
2. Rosen PP, Oberman HA.Tumors of the mammary gland. Amer Registry of Pathology; 1993.
3. Ribli D, Horváth A,Unger Z, Pollner P, Csabai I. Detecting and classifying lesions in mammograms with deep learning. Scientific reports. 2018;15;8(1):1-7.
4. Rooney A. The story of medicine. Arcturus Publishing; 2009 .
5. Ferlay, J., et al. (2013). "GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11
6. Byrne JP. Encyclopedia of pestilence, pandemics and plagues. Medical Education 2008;1100:1500.

7. Silva E, Tan M. The impact of margin status on breast conservation rates. Annals of surgical oncology.2019 ;1;26(12):3931-8.
8. R.L.Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, CA Cancer J. Clin. 69 (1)(2019) 7–34.
9. C.E. DeSantis, J. Ma, M.M. Gaudet, L.A. Newman, K.D. Miller, A. Goding Sauer,Jemal, R.L. Siegel, Breast cancer statistics, 2019, CA Cancer J. Clin. (2019).
10. S.M. Mousavi, K. Hemminki, Cancer incidence, trends, and survival among immigrants to Sweden: a population-based study, Eur. J. Cancer Prev. 24 (Suppl 1)(2015) S1–S63.
11. American Cancer Society Cancer Facts & Figures, (2019) Available from: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2019/cancer-facts-and-figures-2019.pdf.Accessed October 21, 2019.
12. N.N.A. Howlader, M. Krapcho, D. Miller, A. Brest, M. Yu, J. Ruhl, Z. Tatalovich, A. Mariotto, D.R. Lewis, H.S. Chen, E.J. Feuer, K.A. Cronin, SEER Cancer Statistics Review, 1975-2016, National Cancer Institute, Bethesda, MD, 2019 Available from: https://seer.cancer.gov/csr/1975_2016/ , based on November 2018 SEER data submission, posted to the SEER website, April 2019.
13. B.A. Kohler, R.L. Sherman, N. Howlader, A. Jemal, A.B. Ryerson, K.A. Henry, F.P. Boscoe, K.A. Cronin, A. Lake, A.M. Noone, S.J. Henley, C.R. Eheman, R.N. Anderson, L. Penberthy, Annual report to the nation on the status of Cancer, 1975-2011, featuring incidence of breast cancer subtypes by race/ethnicity, poverty, and state, J. Natl. Cancer Inst. 107 (6) (2015) djv048, 1093/jnci/djv048.
14. P.J. Goodwin, V. Stambolic, Impact of the obesity epidemic on cancer, Annu.Rev.Med. 66 (2015) 281–296.
15. H.G. Welch, L.M. Schwartz, S. Woloshin, Are increasing 5-year survival rates evidence of success against cancer? JAMA 283 (22) (2000) 2975–2978.
16. O. Egen, K. Beatty, D.J. Blackley, K. Brown, R. Wykoff, Health and social conditions of the poorest versus wealthiest counties in the United States, Am. J. Public Health107 (1) (2017) 130–135.
17. Sarkar S, Mandal M. Breast cancer: classification based on molecular etiology influencing prognosis and prediction. Breast Cancer-Focusing Tumor Microenvironment, Stem cells and Metastasis. 2011.
18. Ribli D, Horváth A, Unger Z, Pollner P, Csabai I. Detecting and classifying lesions in mammograms with deep learning. Scientific reports. 2018:15;8(1):1-7.
19. Rooney A. The story of medicine. Arcturus Publishing; 2009.
20. Hartmann L. C., Sellers T. A., Frost M. H., Frost M. H., Lingle W. L., Degnim A. C., Ghosh K., Vierkant R. A., Maloney S. D., Pankratz V. S., Hillman D. W., Suman V. J., Blake C., Tlsty T., Vachon C. M. Benign breast disease and the risk of breast cancer. *N Engl J Med*. 2005;353: 229–237.
21. Eberl M. M., Sunga A. Y., Farrell C. D., Mahoney M. C. Patients with a Family History of Cancer: Identification and Management. *JABFM*. 2005; 18:211–17.
22. Breast cancer. emedicine health. 2010. [20 Mar2010]. http://www.emedicinehealth.com /breast_cancer/page2_em.htm .
23. Fletcher S. W. Patient information: Risk factor for breast cancer. Up-To-Date. 2008. Jan 29, [20Mar2010]. http://www.utdol.com/patients/content/topic.do?topicKey=~ _rZvVFHbEjbw .
24. Tiernan A. M. Behavioral risk factor in breast cancer: Can risk be modified? *The Oncologist.* 2003; 8:326–334.
25. Definite breast cancer risks. CancerHelp UK. 2008. Sep 26, [20 Mar 2010]. http://www.cancerhelp.org.uk/type/breast-cancer/about/risks/definite-breast-cancer-risk
26. von Hansemann D (1890) Ueberasymmetrische Zelltheilung in Epithelkrebsen und derenbiologische Bedeutung. *Virchows Arch Path Anat*119: 299-326.
27. Love SM. Dr.Susan Love's breast book. Da Capo Lifelong Books;2015.
28. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer. The Lancet. 1997; 11:350(9084):1047-59.
29. Speroff L, Fritz MA, editors. Clinical gynecologic endocrinology and infertility. lippincott Williams &wilkins; 2005.
30. Schatzker M. The dorito effect: the surprising new truth about food and flavor. Simon and Schuster;2015.

31. Harding F. Breast cancer: cause, prevention, cure. Tekline publishing; 2006.
32. DeVaux RS, Herschkowitz JI. Beyond DNA: the role of epigenetics in the premalignant progression of breast cancer. Journal of mammary gland biology and neoplasia.2018; 1;23(4):223-35.
33. McDivitt RW, Stewart FW, Berg JW.Tumors of the breast.US Department of Defense, Armed Forces Institute of Pathology; 1968.
34. Thiele JA, Bethel K, Králíčková M, Kuhn P. Circulating tumor cells: fluid surrogates of solid tumors. Annual Review of Pathology: Mechanisms of Disease.2017 24;12:419-47
35. Fan SF, Zeng ZY, Peng HW, Guo ZM, Wang SL, Zhang Q. Sentinel lymph node biopsy versus elective neck dissection in patients with cT1-2N0 oral tongue squamous cell carcinoma. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology.2014 ; 1;117(2):186-90.
36. Hassanipour S, Mohammadzadeh M, Mansour-Ghanaei F, Fathalipour M, Joukar F, Salehiniya H, Abdzadeh E, Samadani AA, Nikbakht HA, Arab-Zozani M. The incidence of hepatocellular carcinoma in iran from 1996 to 2016: A systematic review and meta-analysis. Journal of gastrointestinal cancer.2019 ;50(2):193-200.
37. De Decker M, De Schrijver L, Thiessen F, Tondu T, Van Goethem M, Tjalma WA. Breast cancer and fat grafting: efficacy, safety and complications—a systematic review. European Journal of Obstetrics & Gynecology and Reproductive Biology.2016 ;1;207:100-8.
38. Carroll RG, inventor; Care Wise Medical Products Corp, assignee. Apparatus and methods for providing attenuation guidance and tumor targeting for external beam radiation therapy administration. United States patent US 5,813,985. 1998.
39. Fassnacht M, Dekkers OM, Else T, Baudin E, Berruti A, De Krijger RR, Haak HR, Mihai R, Assie G, Terzolo M. European Society of Endocrinology Clinical Practice Guidelines on the management of adrenocortical carcinoma in adults, in collaboration with the European Network for the Study of Adrenal Tumors. European journal of endocrinology.2018;1;179(4): G1-46.
40. Osborne C., Wilson P., Tripathy D. Oncogenes and tumor suppressor genes in breast cancer. Potential diagnostic and therapeutic applications. *The oncologist.* 2004;*4*: 361–377.

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RESEARCH ARTICLE

A study on Student's Engagement in Online Classes using e learning tools in Chennai City

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ABSTRACT

Online learning has become a viral tool during new normal, during this COVID pandemic period. Even though online learning using e resources is preferable with many positive outcomes it reflects negative outcomes while engaging students. The research paper aims to analyze the student's engagement using e learning tools during a pandemic with special reference to Chennai city. The objective of the study is to identify the factors which influence student's engagement and e learning among college goers. It was an empirical study and data was collected using a structured questionnaire with 182 completed ones. The study found that majority prefer online updating and many students prefer online lecture with a ppt to understand concepts. Statistical tools highlights there exist a significant difference between male and female respondents on opinions towards online learning based on different aspects. Mean score concludes that e-learning is more useful during quarantine time for arts course respondents than the science course respondents. The study finally concludes with some suggestions to improve online Learning environment by effectively approaching the student's behavioral, emotional and cognitive factors.

Keywords: e-learning, student's engagement, learning tools

INTRODUCTION

The Covid-19 pandemic has made the students struggle to learn the things related to their subjects from their usual traditional way of classroom learning. Educational institutions were using online methodologies to teach their students. There was a dramatic change in the learning method as almost 90% of the institutions instructed their teachers to go with online learning tools to teach the students. Usage of online resources was better in digitally well developed countries and students were very comfortable with e learning process. In countries like India, Students were engaged with the social media like whatsapp, Facebook, Youtube, etc., and it is new for most of the students to use or to learn the subjects through online learning tools such as GMeet, Google Classroom, Zoom, Webex, Microsoft teams and You Tube. Teachers in India are struggling a lot to get engaged the students to online learning classes as students were not engaged actively or fully due to various reasons. Online learning needs an environment which has to provide the students to get engaged in the online classes without any disturbances. This research paper deals with the students' engagement in online classes and the reasons for not engaging in online classes. The reasons for the engagement and disengagement of the students' in online classes are from three elements namely students, teachers and the environment. Moreover, the study has suggested how to engage students actively using e sources .Students' engagement in online learning can be understood by knowing the factors which influence the students for being engaged and disengaged.

Objectives of the study

To study the demographic profile of the respondents.
To find out the factors which influence the students engagement with respect to e learning.
To study the impact of online learning during COVID-19 lockdown.

Review of Literature

Peter khan et al. (September 2016) has analyzed the student's engagement in online learning environment. The role of reflexivity that the environment under which the student learns through online triggered reflexivity that they have to establish concrete courses of action and sustained practices, to face the uncertainty and complexity in learning through online. They found that the reflexivity of students is framed by the tasks assigned to them and the social relations in the learning environment. There were different dissonances between students learning and the environment. The students need digital communications on line to be promoted to them to resolve such dissonance. To facilitate them, students have been developed with those practices in getting attention to online learning.

Min Hu and Hao Li(2017), in their research "student engagement in Online Learning have explored the engagement of student on online learning through three aspects namely the quantitative methods, for attendance rate, grade, learning time, rate of work completion, Event report; Qualitative methods for students and teachers surveys, self-report, Observations; and mixed methods, for combining both. They additionally found that the student engagement is multi-dimensional which consists of behavior of the student on explicit and psychological behavioral reflections. They have finally arrived at a conclusion that the teachers have to find different measures to make the students to get engaged in the online learning and ensure the learning outcomes.

Donna R.Everett(Now.2015), in the paper "Adding Value: Online student Engagement" has discussed the importance of student engagement in online learning and has reported the eight new developments in online learning. In 2014 ELearning Industry Report, by providing the value of global E-learning industry $56.2 billion and it is been projected to the double on the next year. The report had said that 41.7% of the global fortune 500 companies are using educational technology to instruct employees. The report shows that E-Learning has the power to increase information retention rates by up to 60%(A report by the Research Institute of America). 72% of the companies included in a survey stated that E-Learning helps them to keep up-to-date changes in their industry. The research found that the student engagement in online is based on the tool, device/gadgets they use, the instructional strategies

used by the teachers, the technology adopted for teaching learning, Asynchronous and Synchronous, instructors approach, class and assignment structure, learning support and the environment under which the students are learning. Lily Wong (2013), in the paper titled, "Student Engagement with Online Resources and its impact on Learning Outcomes" Stated that the quality of learning is the outcome of the online resources for learning. Those resources were, Firstly, the students' prior experience, knowledge, conceptions and reasons for studying. Secondly, the students' perceptions of the teaching and learning environment. Thirdly, the type of teaching and learning environment provided. Fourthly, the teacher's pedagogical course knowledge and conceptions of teaching. Fifthly, how the course material is selected, organized, presented and assessed. Lastly, the approaches to learning and studying.

Orna Farrell and James Brunton(2020), in their paper titled " A balancing act: A Window into online student engagement experiences" have studied the student engagement in online learning though the factors which influencing by socio-cultural elements they have included the socio-cultural environment by culture, power, policy and economics. They have made a conceptual framework, in which they have categorized the structural influences, (Institution-Culture, policies, Curriculum, Assessment, Disciplines, Student background, support, Family and life load), Psychological influences(Institutions teaching, staff support, workload and its relationship with students motivation, skills, identity and self efficiency), Proximal consequences(Academic retention, work success, lifelong learning, social citizenship and personal growth). These categorical influences affect the student engagement on their enthusiasm, interest and belongingness. Moreover, it made the students focus on their cognition like deep learning and self regulation. The behavior that would have been influenced by these conceptual frameworks on Time and Effort, interaction and participation.

Gregor Kennedy (2020), in his paper titled," What is student engagement in online learning….and how I know when it is there?" (A Melbourne CSHE discussion paper) had offered three perspectives on student engagement in online learning environment and had provided some sort of advices to educators how it might be promoted? He had came out with three perspectives namely a) An Interaction Perspective, in which the high level of engagement was depend upon the interaction between students and teacher, student and student, and student and the content, b) An Interactive Perspective, in which the use of gadgets by the students for learning and the environment might boost the behavioral and cognitive engagements and it would be beneficial, c) A Learning Design Perspective, in which the online curriculum had designed after careful thinking of the teacher's area of knowledge, content expertise, planned activities of tasks and assignments, etc. A learning design model would be Inquiry-based, Simulation-based and Peer-based learning models that he was suggested. He had arrived at a conclusion that new and timely approach was needed and the material given to the student was important.

Research Methodology

To achieve the research objectives, the researcher used two phases:

Phase One: is on the literature review and concepts to explore the things related to student engagement on online learning during COVID-19 lockdown. Those are the following:

- Social Networking
- Online Applications to Learning
- The mostly used online learning tool
- The positive and negative factors which influence student engagement on online learning
- The drawbacks of social networks on online education.

Phase Two: it is to explore the preference, opinions, attitude, behavior and reasons for engaged and not engaged in online classes by asking questions through google forms and got responses from a structured questionnaire that has been sent as google link. The respondents were college students from the colleges in Chennai, Tamil Nadu. The study has got completely filled responses from 182 students.

Analysis and research findings

Research questions were asked to the respondents through Google form which start with the demographic profiles of the respondents and continued with their preference to learn online, their most engaging activity in online learning, gender-wise opinion, the platform that they have used to learn and ended with the usefulness of online learning during COVID-19 lockdown. To analyze the aforesaid things, the researcher has done a statistical analysis using mean, standard deviation, percentage analysis, t-test etc.

Table: 1 shows the demographic profile of the respondents. The number of respondents for this study is 182. In that 75.3 percent of the respondents are female, 54.4 percent of the respondents are belongs to science course students and 25.8 percent of the respondents are 20 years of age. The demographic profile of the respondents helps the researchers for further analysis of the study. Table: 2 shows the respondents preference to learn and update through online classes. 31.3 percent of respondents are agreed to learn and update through online classes. Only 3 percent strongly disagreed to learn and update through online classes.

Table: 3 shows the most engaging activities that respondents find in online class. Majority of respondents (50 percent) mostly engaged in power point presentation. Least 13.2 percent of respondents are engaged in online internet forum discussion. Table: 4 shows the t test result of opinions towards online learning with gender level. H_0: There is no significant difference between male and female respondents on opinions towards online learning based on different aspects.

As the p value is less than 0.05, the null hypothesis is rejected at a 5 percent level with regard to the statements 'More useful during quarantine period', 'E-learning makes knowledge wider' and 'Satisfied with the online learning'. Hence, there is a significant difference among male and female respondents with regard to above statements. From the mean score, female respondents are more favor to the above statements compare to male respondents. As the p value is greater than 0.05, the null hypothesis is accepted at a 5 percent level with regard to the statements 'Creates confidence', 'Improves self-study skill', Better than the traditional learning', 'Getting all the help that need for online class from teacher' and Innovative method and an effective way of learning'. Hence there is no significance difference between male and female respondents on the above statements.

Table: 5 show the respondents mode of online platform used for their E-learning. Majority 64.8 percent of the respondents used Gmeet platform for their online classes. 24.2 percent of respondents used Zoom platform for their online classes. Least 1.1 percent used webex and YouTube platform for their online classes. Table: 6 show the t test result of course of study and e-learning is more useful during quarantine time. H_0 : There is no significant difference between arts and science course respondents on e-learning is more useful during COVID-19 lockdown.

As p value is less than 0.01, the null hypothesis is rejected at 1 percent level of significance with regard to e-learning is more useful during quarantine time. Hence, there is a significant difference among arts and science course respondents with regard to e-learning are more useful during quarantine time. From the mean score it can be concluded that arts course respondents stated that e-learning is more useful during quarantine time than the science course respondents.

Suggestions to increase online student engagement

The online Learning environment can be made effectively by approaching the student's behavioral, emotional and cognitive factors.

- Behaviorally by making them to follow instruction properly, to participate actively, and to complete their work on time.
- Emotionally by making them feel a part of the class, interacting positively, be alert to them all the time during the class.
- Cognitively by engaging intellectually, more eager to learn, think deeply and ask challenging questions.

- The teacher has to prepare students for the online learning experience with frequent reviews by briefing the online course and also by understanding the expectations of the learners with good presentation with sound learning materials.
- Regular discussions, assessments will give a clear picture in the meanwhile the instructor has to avoid isolation of students and thereby providing a conductive learning environment via playing audios and videos.
- Build a learned mass community by creating discussion forums, team presentations and sharing vital ideas.

CONCLUSION

Being e-learning is a virtual learning there may be lacking in the control of the students as they were from different social learning environments. So, they need to be conveyed with proper instructions on e-learning. Students are showing more interest on e-learning during recent times and they are using different learning tools and each tool is having some unique features in delivering the learning content to the students. It will be good that to reap more benefits of e-learning by engaging the students intellectually to participate in the class make them for being interactive during class timings, etc. A good e-learning environment has to be built by including student's behavioral, emotional and cognitive factors as it affects the students' engagement more.

REFERENCES

1. Carla Hugg Blakey et al., (2020), "Student Perceptions of Engagement in Online Courses: An Exploratory Study", Online Journal of Distance Learning Administration, Vol.22, Pp: 1-11.
2. Orna Farrell at al., (2020), "A balancing act: a window into online student engagement experiences", International Journal of Educational Technology in Higher Education, Vol.17, No.25, Pp: 1-19.
3. Gregor Kennedy (2020), "What is student engagement in online learning…and how do I know when it is there?" (Melbourne CHSE Discussion Paper), Melbourne Centre for the Study of Higher Education, Pp: 1-6.
4. Peter Kahn et al.,(2017) "Understanding student engagement in online learning environments: the role of reflexivity", Education Tech. Research Dev. Vol.65, Pp: 203-218.
5. Min Hu et al.,(2017), "Student Engagement in Online Learning: A Review", International Symposium on Educational Technology, Vol.6, Pp:39-43.
6. Donna R. Everett (2015), "Adding Value: Online Student Engagement", Information Systems Educational Journal, Vol. 13(6), Pp:68-76.
7. Lily Wong (2013), "Student Engagement with Online Resources and Its Impact on Learning Outcomes", Journal of Information Technology Education: Innovations in Practice, Vol.12, Pp:129-146.
8. Lee et al. (2015), "Autonomy Support for Online Students", Tech Trends, Vol.59, No.4, Pp: 54-61.
9. Henrie et al. (2015), " Exploring intensive longitudinal measures of student engagement in blended learning", International Review of Research in Open and Distance Learning, Vol. 16, No.3, Pp: 131-155.
10. Dr. MD B. Sarder (2014), "Improving Student Engagement in Online Courses", American Society for Engineering Education, Vol.15, Pp: 1-15.
11. Lily Wong (2013), "Student Engagement with Online Resources and its Impact on Learning Outcomes", Journal of Information Technology Education: Innovations in Practice, Vol.12, Pp: 129-146.
12. NorzianiDahalan @ Omar et al., (2012), "Student Engagement in Online Learning: Learners Attitude towards E-Mentoring", Procedia – Social and Behavioural Sciences, Vol.67, Pp:464-475.
13. Duncan K et al. (2012), "The effect of synchronous and asynchronous participation on students' performance in online accounting courses", Accounting Education, Vol.21, No.4, Pp: 431-449.
14. www.povertyactionlab.org
15. www.edtechreview.in
16. www.education.uw.edu

Table 1: Demographic profile of the respondents

Demographic factors	Categories	Frequency	Percentage
Gender	Male	45	24.7
	Female	137	75.3
Course of Study	Arts	83	45.6
	Science	99	54.4
Age Group (in years)	18 Years	25	13.7
	19 Years	36	19.8
	20 Years	47	25.8
	21 Years	42	23.1
	22 Years	32	17.6

Table 2: Respondents preferred to learn and update through online classes

Opinions	Frequency	Percentage
Strongly Agree	48	26.4
Agree	57	31.3
Neutral	54	29.7
Disagree	17	9.3
Strongly Disagree	6	3.3
Total	**182**	**100.0**

Table 3: Most engaging activities that respondents find in online class

Options	Frequency	Percent
Online Internet Forum Discussion	24	13.2
Webinar	19	10.4
Power Point presentation	91	50.0
Lectures	48	26.4
Total	182	100.0

Table 4: Respondents Gender and Opinions towards Online Learning – t Test

Opinions towards Online Learning	Gender				t value	P value
	Male		Female			
	Mean	SD	Mean	SD		
Creates confidence	1.62	0.860	1.82	0.907	1.317	0.190
Improves self-study skill	1.60	0.809	1.80	0.821	1.444	0.150
Better than the traditional learning	1.53	0.869	1.50	0.815	0.209	0.835
More useful during quarantine period	1.67	0.707	2.00	0.606	3.067	**0.002**
E-learning makes knowledge wider	1.80	0.842	2.08	0.823	1.971	**0.040**
Getting all the help that need for online class from teacher	1.49	0.727	1.39	0.761	0.733	0.465
Innovative method and an effective way of learning	1.87	0.919	2.02	0.870	1.024	0.307
Satisfied with the online learning	1.58	0.753	1.99	0.827	2.983	**0.033**

Table 5: Mode of Online Platform

Mode of Online Platform	Frequency	Percentage
Zoom	44	24.2
Gmeet	118	64.8
Google Classroom	16	8.8
Webex	2	1.1
Youtube	2	1.1

Table 6: Course of Study and E-Learning is more useful During COVID-19 Lockdown

E-learning is more useful during quarantine time	Course				t value	P value
	Science (99)		Arts (83)			
	Mean	SD	Mean	SD		
	1.00	0.600	1.94	0.924	10.118	<0.001*

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RESEARCH ARTICLE

Effect of Pain Education and Exercises on Sciatic Scoliosis with Low Back Pain."A Case Report

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ABSTRACT

Sciatic scoliosis with low back pain can occur after lumbar disc herniation and is often encountered with difficulty in walking or standing for a longtime and can eventually lead to disability, central sensitization, and low self-esteem. Pain education teaches people more about understanding pain mechanisms to avoid post chronic adverse effects of fear psychosis associated with Pain and fear of movements. A25-year-old male was diagnosed with diffuse lumbar disc prolapse of L4-L5 and L5-S1 with affected bilateral recesses associated with the abutment of bilateral S1 traversing nerve roots. We gave pain education and tailor-made therapeutic exercises to reduce Pain, the stiffness of the trunk and proximal limbs to improve his activity of daily living. Baseline measurements of Pain, range of motion, straight leg raise, Kinesiophobia, and functional ADL assessment were taken pre and post. Outcome measures showed a satisfactory improvement after physical therapy intervention with pain education.

Keywords: Sciatic scoliosis, Low back pain, Pain education, lumbar disc herniation.

INTRODUCTION

Sciatic scoliosis can often result in low back pain after disc herniation with lateral trunk flexion to the opposite side with straightening of the spine in the sagittal plane(1).Patients affected by sciatic scoliosis are often young adults presenting with low back pain and back stiffness. They may progress to developing a scoliotic curve overtime if there is a delay in diagnosis and management(2).Scoliosis is often thought to be the product of a compensatory process to prevent nerve root irritation(3).McKenzie's manual correction and exercise solution may be beneficial; particularly for changes of less than three months(4).Pain education teaches people about the understanding of pain mechanisms and their pain experiences(5).We aimed to study the effect of tailor-made exercises with pain education in this case study.

Case presentation

History

At the outset, it presented as sudden onset of Pain with difficulty in bending forwards, and scoliosis developed later after two months post the first episode of back pain. The Pain was aggravated by bending forwards and running. Later, the patient developed progressive stiffness of lumbar spine, tightness of hamstrings, and worsening in the last six months**. E**ventually, he was confined to bed, unable to walk for short distances with low self-esteem, and worsened daily living activities with lack of proper sleep. He consulted an orthopedician who ordered an x-ray for the lumbar spine and prescribed pregablin and vitanerve injections for one month with no improvement. Later he visited a neurosurgeon who recommended magnetic resonance imaging (MRI) for the Lumbosacral spine which confirmed diffuse disc bulge at L4-L5 and L5-S1 levels. He was prescribed aceclofenac and ryme forte for two months, but symptoms did not improve. Finally, Neurosurgeon referred the patient to Physical Medicine & Rehabilitation department for Physiotherapy consultation and management. He had no previous medical or surgical history.

Physical Therapy Examination

He was referred to us two months post the diagnosis of sciatic scoliosis. His chief complaints were low back pain, sometimes radiating to the left buttock, difficulty walking with a guarded gait, and lots of anxiety with depression. He also complained that he can't sit in a chair for more than 30 minutes, which hampered his studies and was unable to bend forwards with knee straight due to pain and hamstring tightness, which rendered him unable to play outdoor games as he was an athlete before. The straight leg raise test was just 35degrees. We measured range of motion with a goniometer, which was lesser in the left lower limb than the right lower limb ((Table 1), On palpation, we assessed the stiffness of the lower back and gluteal region using Mohanty's flat palpation grades, and it was grade 4(➢Table 2).We used the Visual analog scale for pain score(6), Rolland Morris disability questionnaire to assess disability associated with chronic low back pain(7), Tampa scale for fear of movements(8), WHO-5 quality index for quality of life assessment(9), and Barthel index for functional ADL assessment(10).(➢Table 3).He also had a cautious gait.

Physiotherapy Management

The patient was given detailed information about the intervention, and an informed consent was taken before the start of treatment. The main aim was to improve the patient's functional ability and educate him regarding chronic Pain and central sensitization by focusing on adherence to exercise program and removing negative thoughts about well-being. The treatment plan was scheduled three times a week with a home exercise program. After eight weeks of intense physiotherapy followed by daily home exercises due to covid lockdown restrictions with a total of 24 sessions at Physical Medicine & Rehabilitation outpatient department. The patient received pain education explaining the mechanism of chronic Pain, central sensitization, and fear of movements that can lead to deterioration of health and disability. The initial exercise (butterfly flaps) was done for 30 counts and repeated twice with a gap of 5 seconds in between 2 sets. Rest all exercises were done for ten counts and repeated three times on both sides. All

the exercises were prescribed with brisk walking upto 100meters. Initially, the patient said he was unable to walk even 100 meters without Pain. But was told that he would improve gradually and was advised to do cycling for 500 meters and then to progress it to 1000 meters gradually as tolerated for general warm-up and endurance. Each session lasted for 1 hour and 30 minutes (➢Table 4). Later after 6 weeks he was put on phase 2 of progressive exercise plan which included exercises in sitting, supine lying and standing (➢Table 5).

Measures and outcomes

After 2 months of intense physical therapy, follow up was done, the outcomes showed improvement in pain score, disability score, kinesiophobia and WHO-5 quality index while no difference in Barthel ADL assessment. Also sciatic scoliosis improved. Overall improvement was seen in all outcome measures except ADL assessment (➢Table 3).The patient reported that the moderate low back pain during rising from sleep has ceased, and he could now do a brisk walk which wasn't possible at the start of physical therapy management. In addition he can now do the second stage of progressive exercises which were demonstrated and taught him after completing initial phase exercises (➢Table 4).He sometimes feels stiffness in the back or casual episodes of sour back. In our opinion he is eager and more vigilant now to get back to his normal daily routine. We discussed and explained to him that to be assured that these casual episodes will wean off as soft tissue sensitivity to movements decrease. During the last follow up on 17th may 2021 he had an excellent pain free range of movement in the spine and hips, and now he can touch his toes without Pain and tightness in the hamstrings. This is because of his positive attitude and Adherence to exercise program. We had advised him to try swimming and report to us how he felt after a swim as he mentioned he was a swimmer too in the past, and he was feeling much better after swimming and reported that his endurance and back stiffness have improved.

Study implications

The patient outcomes showed improved pain, disability and quality of life, and straight leg raise. His flexibility of the spine improved and also the scoliotic curve. A case report of manual correction of an acute lumbar lateral shift using McKenzie MDT showed complete recovery of deformity within hours of initial treatment and Pain relieved in 3 days. The athlete was back to routine within three weeks of the rehabilitation program(2).Another case report on lateral lumbar lateral shift was successfully managed by manual therapy and exercises using biofeedback mirror and Swiss ball(4). So we can summarize in conclusion that pain education and tailor-made exercises appear to be most beneficial for improving lateral curvature of the spine and preventing the adverse effects of central sensitization. The patient was very depressed and had a low self- esteem but after treatment he expressed happiness and was more enthusiastic and motivated towards his health with improved confidence and holding high self-esteem.

Institutional study Review Board (ISRB)

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Conflict of interest

Authors declare that there is no conflict of interest

Author contributions

MS, AR conceived and designed the study, MS, UM conducted research, provided research materials, and collected and organized data. MS, FZK, RS wrote initial and final draft of article, and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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REFERENCES

1. Zhu Z, Zhang Y, Li W, Xu L, Jiang E, Qiu Y. Retrospective Review Sciatic Scoliosis Evolution after Lumbar Discectomy: A Comparison Between Adolescents and Young Adults [Internet]. Available from: www.painphysicianjournal.comwww.painphysicianjournal.com
2. Laslett M. Manual Correction of an Acute Lumbar Lateral Shift. J Man Manip Ther. 2009;17(2):78–85.
3. Gillan MGC, Ross JC, McLean IP, Porter RW. The natural history of trunk list, its associated disability and the influence of McKenzie management. Eur Spine J. 1998;7(6):480–3.
4. Peterson S, Laslett M. Alternating lumbar lateral shift: a case report. J Man Manip Ther [Internet]. 2020;00(00):1–8. Available from: https://doi.org/10.1080/10669817.2020.1787747
5. Louw A, Nijs J, Puentedura EJ. A clinical perspective on a pain neuroscience education approach to manual therapy. J Man Manip Ther [Internet]. 2017;25(3):160–8. Available from: http://doi.org/10.1080/10669817.2017.1323699
6. Ciprandi G, Tosca MA, Signori A, Cirillo I. Visual analogue scale assessment of nasal obstruction might define patients candidates to spirometry. Rhinology. 2011;49(3):2001.
7. Stratford PW, Binkley J, Solomon P, Finch E, Gill C MJ. Roland-Morris Low Back Pain And Disability Questionnaire (RMQ). Phys Ther. 1996;76(4):359–65.
8. Tampa Scale for Kinesiophobia. Encycl Pain. 2006;2395–2395.
9. Bech. WHO-5 Well-being Index. DepCare Proj Heal All. 2004;60246.
10. Use T. THE The Barthel ADL Index : Guidelines. 2008;0–1.

Table 1. Range of motion measurements

AROM	Right		Left	
	Before	After	Before	After
Hip Flexion	45	85	35	70
Hip extension	45-0	65-0	35-0	45-0
Hip Abduction	35	45	40	40
Hip adduction	25	25	20	20
Hip external rotation	Normal	Normal	30	40
Hip internal rotation	30	30	30	30
Knee flexion	120	120	120	120
Knee extension	Normal	Normal	Normal	Normal
Ankle "all movements"	Normal	Normal	Normal	Normal

Abbreviation: AROM is active range of motion

Table 2. Mohanty's flat palpation grades

The various layers palpated by the therapist are	Grades
Layer 1= Skin (soft tissue) Layer 2= subcutaneous tissue Layer 3= Superficial muscles Layer 4= Deep muscles with fascia	Grade 1= Adherence of layer 1,2,3 & 4 Grade 2= Adherence of layers 2,3 & 4 Grade 3= Adherence of layers 3 & 4 Grade 4= Toughness in the deep fascia Grade 5= Normal

Table 3. outcome measures

Outcome measure	Before	After
VAS	8/10	3/10
Rolland Morris disability questionnaire	20/24	5/24
Tampa scale	76/100	37/100
WHO-5 quality index	40	84
Barthel index	70/100	70/100
Straight leg raise	30/70	70/70

Table 4 Initial therapeutic exercise program description

Starting Position	Exercise	Exercise Description
Sitting	Butterfly Flaps	Sitting upright with both the knees flexed, hip abducted, sole touching one another. Moving the knees up and down simultaneously (flapping).
	Seated compound stretch for hamstring & latissimus dorsi	Sitting with one knee flexed and the other leg extended (unilateral butterfly pose). Touching the same side hand to leg as the trunk moves in side flexion.
	Sitting Unilateral butterfly dynamic stretch	Sitting in unilateral butterfly pose (as described in the column above) and turn the trunk laterally towards the extended leg and move the trunk neutral to flexion to neutral.
	Sitting straddle stretch	Sitting with both the hips and knees extended. Hip abducted to its maximum. Placing the palm/ elbow (based on their flexibility) flat on the ground.
	Seated mermaid trunk turn	Sitting upright with one knee and hip flexed at right angle while the other hip is internally rotated and knee flexed at 120 degree. Trunk to be flexed forward and then gradually turned into lateral flexion.
Side lying	Side plank dips	Lying down on one side, shoulder abducted at 90 degrees, elbow flexed at right angle. Legs stacked one over the other, trunk to be moved up and down along with the hip going off the floor.

Table 5. Progressive exercise plan description

Starting Position	Exercise	Exercise Description
Sitting	Butterfly flaps	Sitting upright with both the knees flexed, hip abducted, sole touching one another. Moving the knees up and down simultaneously (flapping)
Supine lying	Static Piriformis stretch	Supine lying with one knee flexed straight on the floor, while the opposite leg (ankle)

		is placed over the knee (as in figure of 4 position). Griping the flexed knees thigh and approximating it to the chest. Repeat the same on the other side.
	Unilateral Hamstring stretch	Supine lying with one knee flexed. Wrapping a thin Cloth/ band around the other legs (extended leg) forefoot. Lifting the leg with the assistance of the cloth/ band to its maximum and holding the position. Repeat the same on the other side
	Bilateral dynamic Bridging on a chair	Placing both the legs (calf to feet) on the chair. Hands by the side. Lifting the hip up and bringing it down back to the ground slowly.
	Unilateral Dynamic Bridging on chair	Placing one leg on chair and the other leg suspended up in the air (with hip flexion at 80 degrees). Hands by the side. Lifting the hip off the floor and bringing it back to the ground slowly. Repeat the same on the other side.
	Isometric Quadriceps strengthening	Lying supine with a pillow placed below both the knees. Extending the knee with few seconds hold and relaxing.
	Isometric Hamstring Strengthening	Placing one pillow below both the heels. Dorsi flexing the ankle while extending the knees with few seconds hold and relax.
Standing	Free Squats (full range)	Standing with legs hip-distance apart. Flexing the hip, knee, and ankle to move the butt close to the ground and stand up to starting position.
	Unilateral Squats (beginners level)	Standing with one leg off the floor, flexing the dependant hip and knee to bring the butt in contact with the chair placed behind. And immediately standing up without putting the other leg on the ground. While doing this, the subject is not allowed to use his arms for support. Repeat the same on the other side.

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RESEARCH ARTICLE

Analytical Study on Price Movement of Particular Automobile Stocks using Technical Indicators

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ABSTRACT

This study aims at understanding the price movements of selected automobile stocks- Tata motors, Hero MotoCorp, Maruti Suzuki using Technical Indicators such as Exponential Moving Average, Relative strength Index & Bollinger bands. Technical analysis helps the investors to understand about the price movements and expected trend in future using past prices and volume. The data for this study is collected from authenticated sources such as websites, Newspapers and magazines. The technical indicators will help the traders and investors to forecast the uptrend/downtrend and other trading strategies of selected automobile stocks chosen for this study. It also helps the investors to wisely decide their effective trading strategies, Entry and exit level and price movements of automobile stocks.

Keywords: Technical Analysis, Relative Strength Index, Exponential Moving Average, Bollinger Bands, Automobile stocks.

INTRODUCTION

This study will help the investors understand the price patterns, trending/non-trending zones of chosen automobile stocks using technical analysis. Technical analysis is a trading discipline used to evaluate investments and identify trading opportunities in price trends and patterns seen on charts. Technical analysis, unlike fundamental analysis, which focuses on a company's financials rather than historical price patterns or stock trends, believe the fact that the past trading activity and price changes of a security can be valuable indicators of the security's future price movements. Relative Strength Index, an important technical indicator which helps investors to understand the buy/sell signals at particular period. The RSI oscillates between zero and 100. Traditionally the RSI is considered overbought when above 70 and oversold when below 30. Signals can be generated by looking for divergences and failure swings. RSI can also be used to identify the general trend. The exponential moving average (EMA) is a weighted moving average (WMA) that gives more weighting, or importance, to recent price data than the simple

moving average (SMA) does. The EMA responds more quickly to recent price changes than the SMA. In this paper, the study aims to study the effectiveness of different technical indicators and chart patterns in order to know the price movements of different stocks. Ultimately, the analysis of different stock analysis with different technical indicators will help the investors predict future price patterns, trending zones and will help the investors to have strong buy or sell signals. Hence, technical analysis, though cannot be completely dependent, provides great insights for the traders and investors to take optimum investment decisions. Bollinger bands is a technical tool consisting of There are three lines with a simple moving average (middle band) and an upper and lower band. The upper and lower bands are typically 2 standard deviations +/- from a 20-day simple moving average, but can be modified (Chart 1).

Overview of Automobile Industry

Domestic automobiles production increased at 2.36% CAGR between FY16-20 with 26.36 million vehicles being manufactured in the country in FY20. Overall, domestic automobiles sales increased at 1.29% CAGR between FY16-FY20 with 21.55 million vehicles being sold in FY20. Two wheelers and passenger vehicles dominate the domestic Indian auto market. Passenger car sales are dominated by small and mid-sized cars. Two wheelers and passenger cars accounted for 80.8% and 12.9% market share, respectively, accounting for a combined sale of over 20.1 million vehicles in FY20. Passenger vehicle (PV) sales stood at 3,10,294 units in October 2020, compared with 2,71,737 units in October 2019, registering a 14.19% growth. As per the Federation of Automobile Dealers Associations (FADA), PV sales in November 2020 stood at 2,91,001 units, compared with 2,79,365 units in November 2019, registering a 4.17% growth. Overall, automobile export reached 4.77 million vehicles in FY20, growing at a CAGR of 6.94% during FY16-FY20. Two wheelers made up 73.9% of the vehicles exported, followed by passenger vehicles at 14.2%, three wheelers at 10.5% and commercial vehicles at 1.3%. EV sales, excluding E-rickshaws, in India witnessed a growth of 20% and reached 1.56 lakh units in FY20 driven by two wheelers. The industry has attracted Foreign Direct Investment (FDI) worth US$ 24.53 billion between April 2000 and June 2020, according to the data released by Department for Promotion of Industry and Internal Trade (DPIIT).

Review of Literature

S.Vijai "predictive ability of fundamental and technical analysis", examined the stock market index movement for the period of 2000 – 2005 and conclude that the stock market index movement has a close correlation with 2 major fundamental parameters, namely GDP and FII inflow. Author make a suggestion that model used for predicting the movement in Sensex using technical analysis better compare to fundamental analysis. For the research process he takes a period of 2000-2005. Bhargavi R, Srinivas Gomathy, Anith.R(2017) identified that RSI is one of the most effective technical analysis tools available, it can be effectively used to create a portfolio. Just as it performs well in other stock markets around the world, it also works well in Indian stock market. It has also been found out that P/E ratio better reflects the performance of an organization.

Gupta, (2003) examined the perceptions about the main sources of his worries concerning the stock market. A sample comprise of middle-class household's spread over 21 sates/union territories. The study reveals that the foremost tause of worry for household investors is fraudulent company management and in the second place is too much volatility and in the third place is too much price manipulation. Ugur Sahin, A. Murat Ozbayoglu (2014) found that under good market conditions (trendless or bull market) classic RSI performs well; however, it is vulnerable to trend changes. Bing Anderson and Shuyun Li (2015) Found that for the past decade or so, using the standard configuration of RSI < = 30 and RSI > = 70 as buy or sell threshold, RSI offers no trading profit, but a small loss instead. However, when the buy/sell threshold parameters are altered, to deviate from the combination most commonly used, using RSI as the trading signal still yields profits.

The literature with respect to Bollinger Bands simulations is quite vast. Butler and Kazakov apply swarm optimization techniques to search for optimal Bollinger Band Bollinger parameters. The optimizations are done with respect to the profit and loss of Bollinger Band pairs trading strategies. Similarly, Ni and Zhang use genetic

algorithms to find the optimal Bollinger Band window length and band width jointly. The research regarding variations on Bollinger Bands is less plentiful. M. Hashemi Tilehnouei, Shivaraj (2013) observe that MACD performance in making buy, hold and sell signals is better than RSI Divergence. But we cannot skip the important role of RSI in overbought and oversold signals and simply we can diagnose the price whether is it undervalued or overvalued or with suitable value. Bhargavi R, Srinivas Gumparthi, Anith.R(2017) identified that RSI is one of the most effective technical analysis tools available, it can be effectively used to create a portfolio. Just as it performs well in other stock markets around the world, it also works well in Indian stock market. It has also been found out that P/E ratio better reflects the performance of an organization. Michael R. Melton, Xuan (Susan) Nguyen, Michael Simeone, (2017) "Incorporating technical analysis in undergraduate curricula", the paper is presented to provide support for investment decision-making with the understanding that no one technical analysis technique should ever stand alone. Only when used with other technical indicators – in conjunction with fundamental analysis – can a student come to an accurate buy or sell decision.

Objectives of the Study

- To analyse the effectiveness of technical indicators in predicting future price trend patterns
- To understand the price behaviour of automobile stocks contributing to Nifty Auto price movement.
- To create trading strategies of entry and exit levels of the selected automobile stocks
- To make investors aware about the buy and sell signals for future trading
- To understand about the stocks are in trending/non-trending zones for creating optimum investment decisions.
- To know the divergence areas of chosen automobile stocks using technical indicator such as Relative strength Index, Exponential moving average& Bollinger bands.

RESEARCH METHODOLOGY

Research is a systematic and continues method of defining a problem, collecting the facts and analysing them, reaching conclusion forming generalizations. Research methodology is a way to systematically solve the problem. It may be understood has a science of studying how research is done scientifically.

Research Design: A research design is the arrangement of condition for collection and analysis of data in a manner that aims to combine relevance to the research purpose with economy in procedure. It is the conceptual structure within which research is conducted. Analytical Research is used in this study.

Sources of Data: The main sources of data for the present study used are secondary in nature. Secondary data consists of information that already exists somewhere and has been collected for specific purpose in the study. The source of secondary data is collected from Company records, Newspaper, Company websites, NSE. Share price and nifty index for selected companies are collected from www.nseindia.com.

Period of study: The period of study is conducted for a period of 1 year (01/05/2020-30/04/2021)

Tools used for Analysis: Statistical tools are used to analyse Indian stock market using past data and information.

Relative strength Index (RSI)

Relative Strength Index (RSI) is a momentum oscillator that measures the speed and change of price movements. RSI oscillates between zero and 100. When RSI of share is lies between 30 and 70 investors can hold share and if it RSI crosses 70 there may be downturn and it is time to sell.

RSI = 100 – 100/ 1 + RS

RS = Average Gain / Average Loss Average Gain= [(previous Average Gain) x13 + current Gain] / 14. Average Loss = [(previous Average Loss) x 13 + current Loss] / 14.

The standard is to use 14 periods to calculate the initial RSI value. For example, imagine the market closed higher seven out of the past 14 days with an average gain of 1%. The remaining seven days all closed lower with an average

loss of -0.8%. Bullish divergence occurs when the RSI creates an oversold reading followed by a higher low that matches correspondingly lower lows in the price. This indicates rising bullish momentum, and a break above oversold territory could be used to trigger a new long position. A bearish divergence occurs when the RSI creates an overbought reading followed by a lower high that matches corresponding higher highs on the price.

Exponential Moving Average

The exponential moving average (EMA) is a weighted moving average (WMA) that gives more weighting, or importance, to recent price data than the simple moving average (SMA) does. The EMA responds more quickly to recent price changes than the SMA.

$EMA=Price\ (t) \times k+EMA(y) \times (1-k)$

Where:

t=today

y=yesterday

N=number of days in EMA

$k=2\div (N+1)$

To construct a moving average ribbon, simply plot a large number of moving averages of varying time period lengths on a price chart at the same time. Common parameters include eight or more moving averages and intervals that range from a two-day moving average to a 200- or 400-day moving average.

Bollinger Bands

The purpose of Bollinger Band is to determine high and low of stocks. Prices of stocks are high at upper band and low at lower band. Investor can take systematic trading decision by recognise the pattern of the stock. There are three curves drawn in Bollinger Band. The upper band and lower band are simple moving average and middle band is intermediate band. The volatility is determined by interval between upper band, lower band and middle band. The default parameters are 20 periods and two standard deviations.

Correlation

The correlation is one of the most common and most useful statistics. A correlation is a single number that describes the degree of relationship between two variables. Correlation is a tool to identify the relationship level between selected variable. Correlation analysis helps in determining the degree to which the variables are related to each other. Correlation may be positive or negative. When the two variables are moves in the same direction it is called as positive correlation. When the two variables are moves in the opposite direction it is called as negative correlation.

Data Analysis and Interpretation

Bollinger Bands

Tata Motors: Tata Motors Limited is an Indian multinational automotive manufacturing company, part of Tata Group, an Indian conglomerate. Its products include passenger cars, trucks, vans, coaches, buses, sports cars, construction equipment and military vehicles. The above chart 3 clearly explains that tata motors stock is oversold during October 15, 2020 where the price moves closer to the lower band indicating strong buy signal whereas during 05th Feb, 2021 the stock prices moves closer to the upper band indicating sell signal. However currently, the stock is moving sideways where the investors can do partial selling.

Relative Strength Index

Hero MotoCorp: Hero MotoCorp Limited is the world's largest manufacturer of two-wheelers. The company has four manufacturing facilities namely Dharuhera and Gurgaon in Haryana Haridwar in Uttarakhand and Neemrana in Rajasthan. The above chart 4 of Hero MotoCorp clearly depicts that the stock price has been overbought, above the level of 70 in RSI Index during 26th October 2020 and the stock prices been in oversold region as there were concrete efforts made by the sellers for the downtrend during 09th April, 2021 showing sell signal.

Exponential Moving Average

Maruti Suzuki: The Exponential moving average of 21 days clearly shows that the stock prices are below the 21 day EMA depicting downtrend and there was a steady increase in stock price from the month of october witnessing bullish view. The chart 5 indicates that at the initial period of 2020 witness a stern downtrend where the prices are below 21 day EMA and there was a steep fall in share prices of Maruti suzuki until April end showing a buy signal and then the prices started moving above 21 day EMA clearly depicting bullish view.

Correlation

Correlation is a tool to identify the relationship level between selected variable. Here the researcher made an attempt to identify the relationship level between share prices of Selected Companies in Automobile Industry and NIFTY Auto Price movement.

For this purpose of this study the researcher formulated hypothesis is;

Ho: There is no relationship between share price movement of particular Companies in Automobile Industry and the movement of NIFTY Autovalue.

H1: There is a close relationship between share price movement of particular companies in Automobile Industry and the movement of NIFTY Auto value.

Findings

Tata Motors

- Bollinger bands indicator shows a sidewards momentum and in a trending zone where the moving average crossover is slightly bearish.
- Mild Bearish view is indicated through charts where the investors can buy and accumulate Tata motors sharesin the short run and long run
- In terms of Correlation between Nifty Auto and Tata Motors share price movement, it is seen as 93.2% positively correlated which clearly depicts that the stock strongly represents the price movement of automobile industry

Hero MotoCorp

- The current RSI indicator shows that the share price of Hero MotoCorp is trending in a downtrend and it is seen at a level of 30-40 where the moving average crossover are neutral.
- The investors can buy and accumulate shares in the short run and the RSI and EMA clearly shows that is the buy signal
- In terms of Correlation between Nifty Auto and Hero MotoCorp share price movement, it is seen as 83.4% positively correlated.

Maruti Suzuki

- The technical charts of EMA clearly shows that there is an uptrend momentum and there is a Mild Bullish view.
- The analysis depicts that investors can buy the shares however moving average crossovers are neutral.
- In terms of Correlation between Nifty Auto and Maruti Suzuki share price movement, it is seen as 83.4% positively correlated.

Suggestions

- The investors should enter the stock markets with basic information, good knowledge and thorough discipline
- The investors can make effective decisions through technical analysis helping to analyse the stock price movements in order to avoid any losses
- The Bollinger bands, RSI Index and EMS shows that with the selected stocks investors can buy and accumulate the shares in the short and the long run depending upon the momentum of the share prices.
- The relationship between NIFTY Auto movement and the selected auto stocks shows a positive correlation where the investors can earn in these to earn profits.

CONCLUSION

This paper examined the price trends of different automobile stocks with the help of technical indicators such as Bollinger Bands, RSI Index and Exponential Moving Average where we were able to find buying and selling signals, Uptrend/Downtrend momentum, Divergences. This helps the investors make optimum investment decisions. Technical analysts aim to use past performance to predict future market behaviour, helping the investors to trade with adequate information of price movements. It also helps the investors to understand the entry and exit levels of potential trades. Hence this paper helps the investor to identify the trending or non-trending stocks, future price movements which helps them to make effective investment decisions.

REFERENCES

1. Terence Tai-Leung Chong, Wing-Kam Ng, Venus Khim-Sen Liew(2014),"Revisiting the Performance of MACD and RSI Oscillators", Journal of Risk and Financial Management, vol.7, pg.1-12.
2. Prasanna Chandra, "Financial Management-Theory and Practice", Tata Mc Graw Hill, International Edition, 5th edition, 2000.
3. Rena Baral, Abhishek Kumar Chintu (2013), "Study of Technical Analysis for Finding Buying and Selling Signal in Stock Market Through Technical Indicators", International Journal of Entrepreneurship & Business Environment Perspectives, Volume 2, Number 1, pg.288-296.
4. Jinwook Lee, JoonheeLee, Andras Prekopa,"Price-Bands: A Technical Toolfor Stock Trading", Rutcor Research Report, RRR 8-2013, Aug 21, 2013
5. C. Boobalan, "Technical Analysis in Select Stocks of Indian Companies", International Journal of Business and Administration Research Review, Vol.2, Issue.4, Jan-March, 2014
6. Renaud Beaupain, Lei Meng, Romain Belair (2010), "The Impact of Volatility on the Implementation of RSI", Insurance Markets and Companies: Analyses and Actuarial Computations, Vol. 1, Issue 3, pp. 73-78.
7. www.nseindia.com
8. www.eqsis.com
9. www.investopedia.com
10. https://www.moneycontrol.com/india/stockpricequote/banks
11. https://www.traderscockpit.com/?pageView=rsi-indicator-rsi-chart&type
12. www.onlinetradingconcepts.com

Table 1: Table showing Exponential moving average of Maruti Suzuki

Key Moving average of Maruti Suzuki

EMA	Value	Signal	CO Type	Analysis
EMA 5	6631.5	Bullish	Bullish	Price up, Moving Average Trending Up nicely, Buy
EMA 10	6617.15	Bullish	Bullish	Price Trending up, Moving Average Trending getting flat and a Bullish Crossover
EMA 15	6634.11	Mild Bullish	Bullish	Mild Price up, Mild MA Uptrend, Mild Buy
EMA 20	6663.62	Mild Bullish	Bullish	Mild Price up, Mild MA Uptrend, Mild Buy
EMA 50	6872.82	Mild Bearish	NA	Price Abv MA, but MA Not growing
EMA 100	7020.34	Mild Bearish	NA	Mild Price Down, Mild MA Downtrend, Mild Sell
EMA 200	6962.94	Bearish	NA	Price Trending down, Moving Average Trending getting flat and a Bearish Crossover

Tata Motors

Correlations

		Nifty auto	Tata motors
Nifty auto	Pearson Correlation	1	.932**
	Sig. (2-tailed)		.000
	N	250	250
Tata motors	Pearson Correlation	.932**	1
	Sig. (2-tailed)	.000	
	N	250	250

**. Correlation is significant at the 0.01 level (2-tailed).

Hero MotoCorp

Correlations

		Nifty auto	Hero MotoCorp
Nifty auto	Pearson Correlation	1	.859**
	Sig. (2-tailed)		.000
	N	250	250
Hero MotoCorp	Pearson Correlation	.859**	1
	Sig. (2-tailed)	.000	
	N	250	250

**. Correlation is significant at the 0.01 level (2-tailed).

Maruti Suzuki

Correlations

		Nifty auto	Maruti Suzuki
Nifty auto	Pearson Correlation	1	.834**
	Sig. (2-tailed)		.000
	N	250	250
Maruti Suzuki	Pearson Correlation	.834**	1
	Sig. (2-tailed)	.000	
	N	250	250

**. Correlation is significant at the 0.01 level (2-tailed).

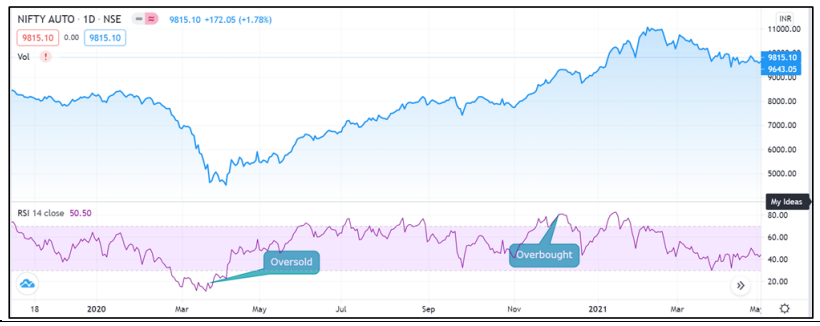

Chart 1: Chart showing a sample of RSI index with overbought and oversold region of automobile industry

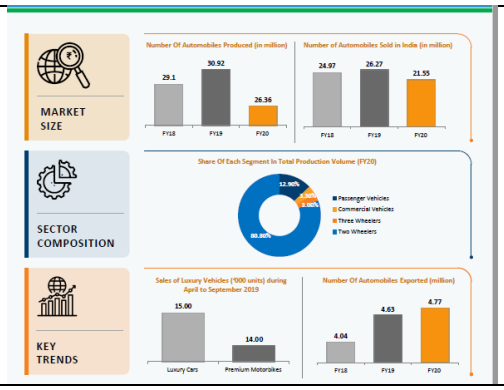

Chart 2: Chart showing market size, sectoral composition & key trends of automobile industry, Source: www.ibef.org

Chart 3: Chart showing overbought and oversold regions of Tata Motors using Bollinger Bands

Chart 4: Chart showing overbought and oversold region of Hero MotoCorp using RSI

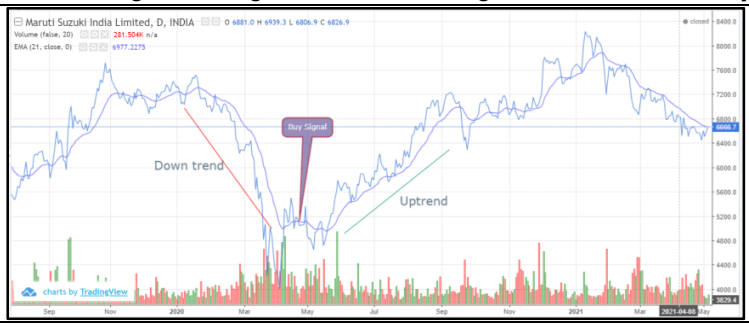

Chart 5: Chart showing uptrend and downtrend of Maruti Suzuki from 2020 to 2021

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RESEARCH ARTICLE

Electrochemical Performance of TiO_2 Nanoparticles Embedded Surface of Smart Coating

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ABSTRACT

Recently, advancement in the nanomaterials is leading towards the exposure in different field such as industry, medical, food technology, forensic, and environment. The studies for the release of nanomaterials are currently limited. Presently, no conventional techniques are available for studying the concentration of released nanoparticles in the environment. Nowadays, nanoparticle coating is available for the different surfaces, due to their exceptional characteristics such as high sensitivity, selectivity, and electrical conductivity. Conventionally, in-use techniques available for nano-coating characterization on the surface include Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), Atomic force microscopy (AFM), Transmission electron microscopy (TEM), Ultraviolet-visible spectroscopy (UV), and X-ray fluorescence (XRF). But these techniques have some limitations such as they require technical person for operation, expensive equipment, and are time-consuming. Recently, electrochemical studies are emerging in different fields because they are less expensive, low cost and are user friendly. The present work shows studies for non-coated and Titanium oxide (TiO_2) nanoparticle coating on the surface of automotive paints with electrochemical techniques such as Cyclic voltammogram (CV), Differential pulse voltammetry (DPV), and Electrochemical impedance spectroscopy (EIS). The result obtained during electrochemical studies clearly differentiates both the samples providing rapid results with lower cost.

Keywords: Impedance; nanomaterials; electrochemical; titanium oxide

INTRODUCTION

During road accident's investigation of automobile paint is a critical issue. The information in an investigation can be retrieved from the evidence present on spot in the form of glass particles, automotive paint chips, foot and finger prints, tool marks, paint stains, gun powder or others. These trace evidences plays an important role in relating criminals with the crime spot in cases especially hit and run cases, road accidents, and robbery [1]. The automotive paint facilitates different properties like decoration, protecting the surface from the surrounding environment (ultraviolet rays, corrosion, surface damage with sharp tool), and functional coating. The paint on the automotive surface is a composition of three layers primer, base coat, and clear coat. The flakes of the paint can be characterized based on the texture, colour and structure of its surface. The chemical composition of paint plays a challenging role for qualitative investigation in forensic science presently microscopic and spectrometric techniques are used for these studies [1,2]. Presently, nanotechnology is an emerging and rapidly developing field hence, an increase in the commercial application of nanoparticles has been reported at a higher rate. In recent years, the behaviour and properties of nanomaterials have been studied in different fields such as-medicine, environment, industry, and food. The engineered nanomaterials (ENM) have been incorporated for several processes such as transformation reactions, agglomeration, and dissolution [3,4]. In the last 2 decades, studies have been reported describing the presence of nanoparticles (NPs) and colloids in the environment. NPs are defined in a dimension range below 100 nm (>100 nm), placing them in a domain of colloidal particles [5,6]. The properties of NPs are distinct, depending on their chemical structure and the synthesis processes. The process of molecules exchange and their attachment on the surface clearly depends on the chemistry of composited elements. They are the composition of complex mixtures different composition of elements in NPs shows their unique and different properties such as enhanced electron mobility and surface to area volume which provides higher conductivity or sensitivity to the system.

The surface of nanomaterials can be functionalized using polymers, metal ions, surfactants, and small molecules [7]. A lot of improvement in different surface material has been observed due to the incorporation of nano-coating. The purpose of nano-coating is mainly for protecting the surface of the substrate, their decoration, and sometimes to hide the substandard substrate material. Nano-material has been used to improve the attributes of the surface with different properties like ultra-violet protection, anti-bacterial, corrosion-resistant, and self-cleaning. The material surface coated with distinct NPs can be examined based on [8-16] the constraint such as size, structure, elemental composition, and morphology. Conventionally, available techniques for these parameter analyses involve Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), Ultraviolet-visible (UV) spectroscopy, and X-Ray Diffraction (XRD) [17]. But these equipments possess some limitations like very expensive and sophisticated so analysis of every sample requires more time, and also require an upskilled technician for operation [18]. Titanium oxide (TiO_2) NPs are oxide semi-conductors they have been reported as promising NPs for various applications due to their low cost, photo and long-term stability, chemical inertness, strong oxidizing power and mechanical properties [19,20]. Therefore, in the presented work TiO_2 nanoparticles were used for surface coating. The presented work describes a comparison of Tio_2 NPs coated and non-coated automobile paint using surface characterization and electrochemical techniques.

MATERIALS AND METHODS

Chemicals and equipment's

Ethanol, acetone, ferrocyanide, and ferricyanide of high purity were purchased from Sigma-Aldrich. Aluminium sheets were procured from the local vendor. Distilled water (DW) was used while carrying out the experiments. All the apparatus were autoclaved before performing experiments. The TiO_2 NPs based coating was procured from DD BIOINFOTECH, New Delhi, India. The electrochemical studies were carried out on potentiostat (SP-150, Biologics

with EC-Lab software). The surface coated with TiO_2 nanomaterial was characterized by distinct techniques includes SEM, AFM, X-ray fluorescence (XRF) spectroscopy, and dynamic light scattering (DLS) at Amity University, Noida, Uttar Pradesh, India.

Sample Preparation

The sample for the experiment was prepared while taking two aluminium sheets which were coated with white paint and kept in polypropylene sheets at room temperature for drying. After one month, one sheet was further coated with TiO_2 NPs based commercially available PROWIN HEALTH smart coating using an electrostatic machine and dried at room temperature. Whereas the other sheet was kept with non Tio_2 NPs coating, flakes from both the sheets were removed and dissolved in acetone overnight. Hence, the solution obtained was used for further characterization and electrochemical studies (Scheme 1).

RESULTS AND DISCUSSION

Sample characterization

The elemental composition of the sample was studied with XRF using an XRF spectrometer (Shimadzu ED7000). Fig. 1 (a) shows the XRF data for poly propylene sheet in this sample Ti was not detected whereas Fig. 1 (b) shows XRF data for poly propylene sheet coated with Tio_2 NPs, 23.9 % of Ti was present in the sample. Further, both (Fig. 1 (c), (d)) white colour paint sample in flake form (coated with nanoparticles and without coating) were analysed with XRF determine the presence of TiO_2 a white pigment, because titanium oxide is present in the form of pigment in the paint. The result of analysis shows that titanium-based pigment was present in both samples. It also shows that the above two samples cannot be differentiated with XRF results as the base paint was having Ti-based pigment.

Scanning electron microscopy analysis was done for surface characterization of the TiO_2 nano-coated flake. The characterization was performed at AIARS, Amity University, Noida, UP, India. The samples were coated with gold-palladium by sputtering to make them conductive before analysis. In Fig. 2 (a) a spherical shape structures on the surface confirms the presence of TiO_2 nanoparticles attached to the sample. The SEM micrograph describes TiO_2 NPs size below 100 nm. For the size distribution of the particles, the DLS technique was performed at AINT, Amity University Noida (Zetasizer Nano-S90, Malvern Panalytical). Fig. 2 (b) shows the size distribution intensity for the two samples. For non-coated surface the Z- average of particles was recorded as 225 nm whereas, for NPs coated surface the Z- average of particles was 119.2 nm and it was also observed from the distribution that the size of some particles is below 100 nm. The topography and surface roughness of the sample was analyzed with AFM. Fig. 2 (c) shows a 3-D image of AFM analysis for the surface of non-coated paint whereas Fig. 2 (d) describes the 3-D image of AFM for TiO_2 NPs coated paint. The results of AFM clearly showing the presence of spherical particles of nanometer size in the sample taken from Tio_2 NPs coated paint.

Electrochemical studies for modified paint samples

Cyclic voltammetry (CV) measurements

Cyclic voltammetry measurements were recorded in a potential range from -0.5 V to +0.5 V in 5 mM ferrocyanide-ferricyanide mediator solution at a scan rate of 20 mV/s. Fig. 3 shows the results of CV study for TiO_2 NPs coated and non-coated paint surface. The oxidation peak for NPs coated sample with 1 mL of solution was observed at 0.65 mA and gradually decreases with the increasing volume, at 2 ml the oxidation peak was observed at 0.57 mA, whereas, the oxidation peak observed for non-coated TiO_2 surface was at 0.5 mA for 1 mL and decreases on further increasing the volume of the solution, with 2 mL it was observed at 0.45 mA. The above result shows that the oxidation peak is appreciably higher in the sample with NPs coated surface, whereas low oxidation peak was observed with non-coated NPs surface with the same sample volume this is because of higher electron mobility due to the presence of NPs on the surface. Further, it was observed that as we increase the sample volume, the peak of the oxidation current

decreases in both cases (NPs coated and non-coated). This condition arises due to the increase in interference caused by the elemental composition of the sample.

Electrochemical Impedance Spectroscopy (EIS) studies

Fig. 4 shows the Nyquist plot, semi-circle shows charge-transfer resistance (Rct) value at higher frequency whereas linear part shows the diffusion process at lower frequencies. The Rct value observed for non-coated surface was higher than the Rct value of TiO_2 NPs coated surface. It shows higher resistivity for non-coated surface whereas due to the excellent properties of NPs the surface is less resistive and highly conductive.

Differential pulse voltammetry (DPV) measurements

For studying the current difference for both samples DPV measurements were performed. Fig. 5 shows the DPV graphs plotted for TiO_2 NPs coated and non-coated surface (i) TiO_2 coated with 1 mL generated higher current of about 70 µA, (ii) when the volume of the solution was increased with 2 mL a gradual decline in the current was observed at 63 µA. (iii) for non-coated surface with 1 mL the current observed was 55 µA and further decreases with the increasing volume, (iv) the current value at 2 mL was obtained around 49 µA. The current value obtained for TiO_2 NPs coated surface was higher than the non-coated surface. A difference of 15 µA was observed between TiO_2 NPs coated and non-coated surface, the bar graph in Fig. 6 shows a clear difference for both the cases.

CONCLUSION

In the present work, electrochemical studies were performed using cyclic voltammetry, electrochemical impedance spectroscopy, and differential pulse voltammetry techniques for TiO_2 NPs coated and non-coated surface for understanding the role of TiO_2 nanoparticles on a surface for the current generation. In the study it was observed that current generated for TiO_2 NPs coated surface was higher than non-coated surface due to the presence of nanoparticles on the surface provide higher electron mobility, therefore, allowing enhancement in the current generation in an electrochemical cell. The result shows that this technique can be very useful for different applications for environmental, industry, and paint sample analysis in forensic research. Due to several advantages of the electrochemical studies as they are less time consuming, low cost, provide highly sensitive data and are user friendly.

ACKNOWLEDGEMENT

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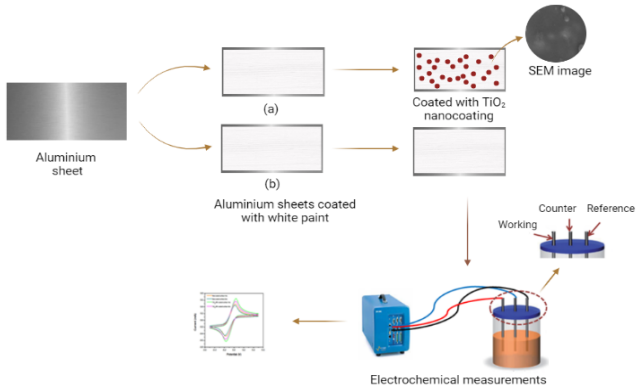
CONFLICTS OF INTEREST

The author declares that there is no competing or financial interest in the submitted manuscript.

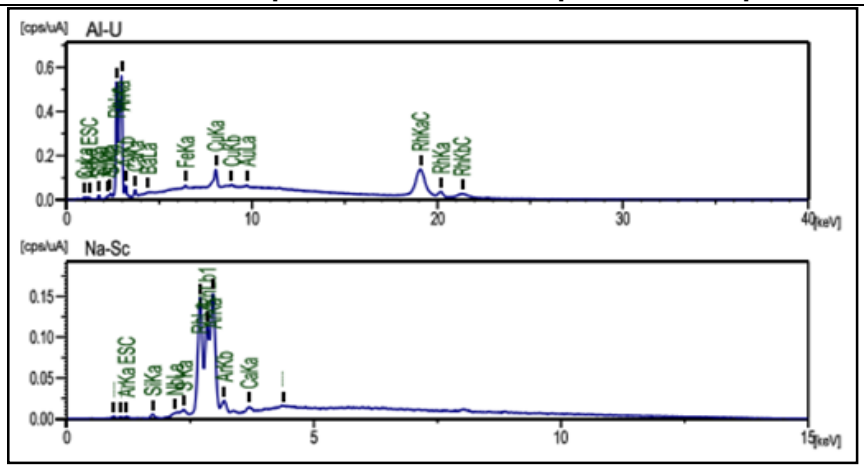
REFERENCES

1. Verma, P.; Kaur, N. and Soni, P. Forensic evaluation and comparison of Indian automobile paint chips using FTIR. Materials Today: Proceedings 2020, 33, pp.1727-1732.
2. Xu, Z.; Anyasodor, G. and Qin, Y. Painting of aluminium panels–state of the art and development issues. In MATEC Web of Conferences (Vol. 21, p. 05012). 2015, EDP Sciences.
3. Feitshans, I.L. Global Health Impacts of Nanotechnology Law: A Tool for Stakeholder Engagement. 2018, CRC Press.

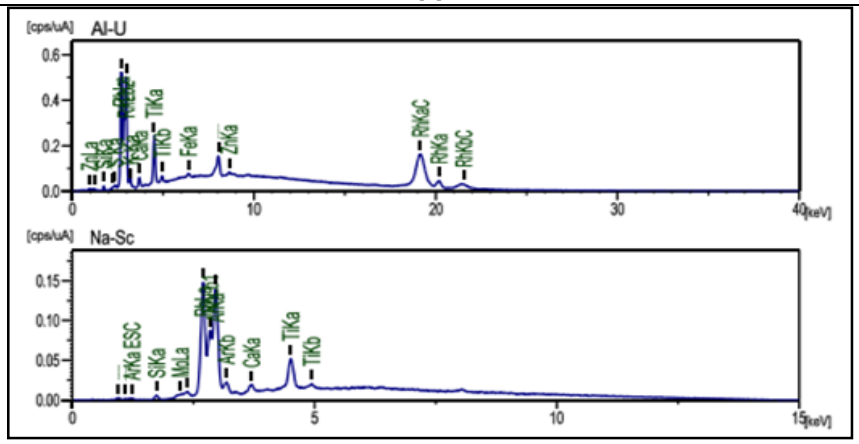
4. Klaine, S.J.; Koelmans, A.A.; Horne, N.; Carley, S.; Handy, R.D.; Kapustka, L.; Nowack, B; and von der Kammer, F. Paradigms to assess the environmental impact of manufactured nanomaterials. Environmental Toxicology and Chemistry, 2012, 31, pp.3-14.
5. Azimzada, A.; Farner, J. M.; Jreije, I.; Hadioui, M.; Liu-Kang, C.; Tufenkji, N.; Shaw, P.; Wilkinson, K.J. Single-and multi-element quantification and characterization of TiO2 nanoparticles released from outdoor stains and paints, Frontiers in Environmental Science, 2020. doi: https://doi.org/10.3389/fenvs.2020.00091.
6. Nored, A.W.; Chalbot, M. C. G.; Kavouras, I. G. Characterization of paint dust aerosol generated from mechanical abrasion of TiO2-containing paints, Journal of occupational and environmental hygiene, 2018,15,629-640.
7. Islam, M.T.; Dominguez, A.; Turley, R.S.; Kim, H.; Sultana, K.A.; Shuvo, M.A.I.; Alvarado-Tenorio, B.; Montes, M.O.; Lin, Y.; Gardea-Torresdey, J.; Noveron, J.C.; Development of photocatalytic paint based on TiO2 and photopolymer resin for the degradation of organic pollutants in water. Science of The Total Environment, 2020, 704, p.135406.
8. Sakinah, S. Z. A.; Azmi, W. H.; Alias, J. Characterization of TiO2 nanopaint for automotive application, In IOP Conference Series: Materials Science and Engineering, IOP Publishing, 2020, 863, 012053.
9. Ferreira, K.B.; Oliveira, A. G. G.; Gonçalves, A. S.; Gomes, J. A. Evaluation of Hyperspectral Imaging Visible/Near Infrared Spectroscopy as a forensic tool for automotive paint distinction, Forensic Chemistry, 2017, 5, 46-52.
10. Khanna, A. S. Nanotechnology in high performance paint coatings, Asian J. Exp. Sci, 2008, 21, 25-32.
11. Solano, R.; Patiño-Ruiz, D.; Herrera, A. Preparation of modified paints with nano-structured additives and its potential applications, Nanomaterials and Nanotechnology, 2020, 10. doi: https://doi.org/10.1177/1847980420909188.
12. Hajipour, M.J.; Fromm, K. M.; Ashkarran, A.A.; de Aberasturi, D.J.; de Larramendi, I.R.; Rojo, T.; Serpooshan, V.; Parak, W.J.; Mahmoudi, M. Antibacterial properties of nanoparticles, Trends in biotechnology, 2013, 30, 499-511.
13. Kabeel, A. E.; Sathyamurthy, R.; Sharshir, S. W.; Muthumanokar, A.; Panchal, H.; Prakash, N.; Prasad, C.; Nandakumar, S.; El Kady, M.S. Effect of water depth on a novel absorber plate of pyramid solar still coated with TiO2 nano black paint, Journal of Cleaner Production, 2019, 213, 185-191.
14. Manixay, S.; Delaby, S.; Gaie-Levrel, F.; Wiart, M.; Motzkus, C.; Bencsik, A. In vivo evaluation of the potential neurotoxicity of aerosols released from mechanical stress of nano-TiO2 additived paints in mice chronically exposed by inhalation, In Journal of Physics: Conference Series, 2017, 838, 012025.
15. Bhuvaneshwari,B.; Vivekananthan,S.; Sathiyan,G.; Palani,G.S.; Iyer,N.R.; Rai,P.K.; Mondal,K.; Gupta,R.K. Doping engineering of V-TiO2 for its use as corrosion inhibitor, Journal of Alloys and Compounds,2020, 816, 152545.
16. Manixay, S.; Delaby, S.; Gaie-Levrel, F.; Wiart, M.; Motzkus, C.; Bencsik, A. In vivo evaluation of the potential neurotoxicity of aerosols released from mechanical stress of nano-TiO2 additived paints in mice chronically exposed by inhalation, In Journal of Physics: Conference Series, 2017, 838, 012025.
17. Ali, I.; Muhyuddin, M.; Mullani, N.; Kim, D. W.; Kim, D. H.; Basit, M. A.; Park, T. J. Modernized H2S-treatment of TiO2 nanoparticles: Improving quantum-dot deposition for enhanced photocatalytic performance, Current Applied Physics, 2020, 20, 384-390.
18. Lambert, D.; Muehlethaler, C.; Esseiva, P.; Massonnet, G. Combining spectroscopic data in the forensic analysis of paint: Application of a multiblock technique as chemometric tool, Forensic science international, 2016, 263, 39-47.
19. Schiavello, M. (Ed.). Photoelectrochemistry, Photocatalysis and Photoreactors Fundamentals and Developments, Springer Science & Business Media, 2013, 146.
20. Anirudh, K. V. S.; Bottu, M. V.; Sarvamangala, D. Production of TiO2 nanoparticles by green and chemical synthesis—a short review, Int J Sci Eng Res, 2018, 9, 1633-1648.



Scheme 1: Graphical illustration for experimental setup



(a)



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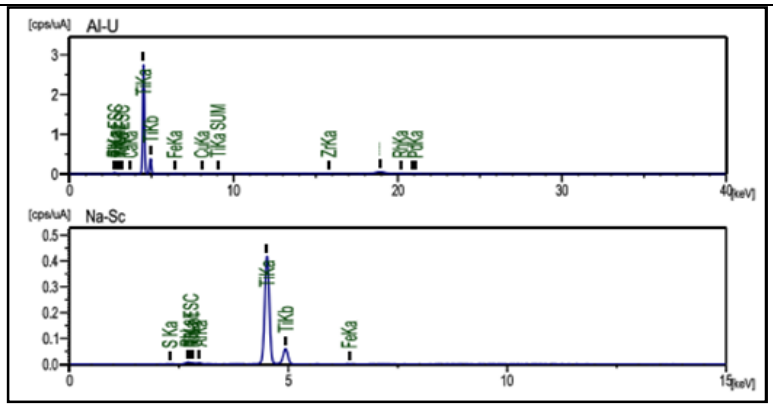

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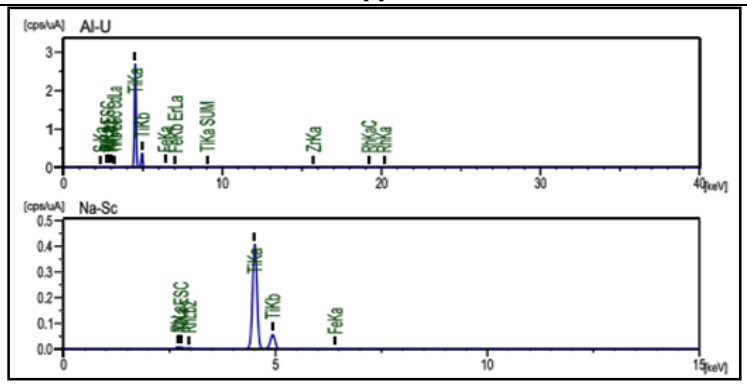

(d)

Figure 1:XRF studies for a) polypropylene sheet, b) TiO_2 NPs coated polypropylene sheet, c) Paint surface without TiO_2 NPs coating, d) Paint surface with TiO_2 NPs coating

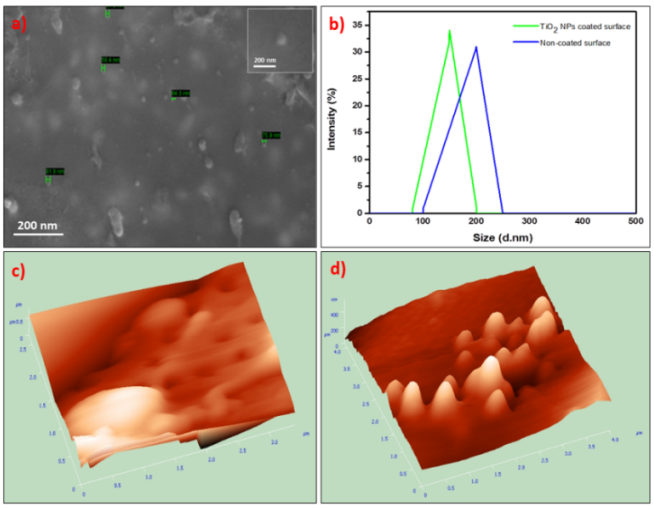

Figure 2: a) SEM image for TiO_2 NPs coated surface; b) DLS of TiO_2 NPs coated surface; c) 3-D image of AFM for non-coated surface; d) 3-D image of AFM for TiO_2 nanoparticles coated surface

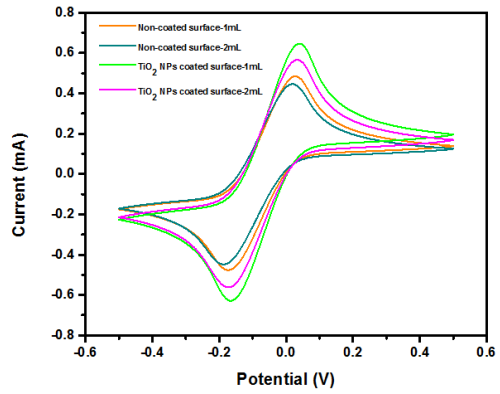

Figure 3: Cyclic voltammetry measurement for non-coated and TiO_2 NPs coated surface

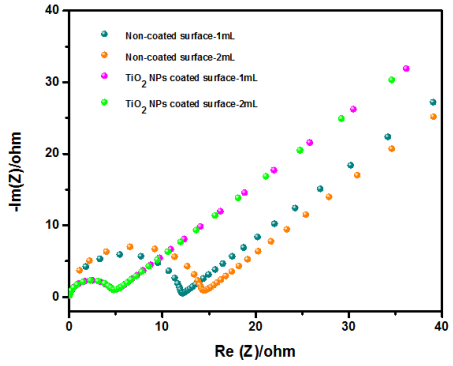

Figure 4: Electrochemical impedance spectroscopy measurement for non-coated and TiO_2 NPs coated surface

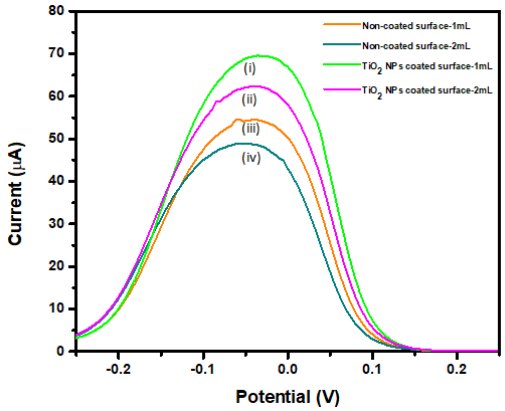

Figure 5: Differential pulse voltammetry measurement for non-coated and TiO_2 NPs coated surface

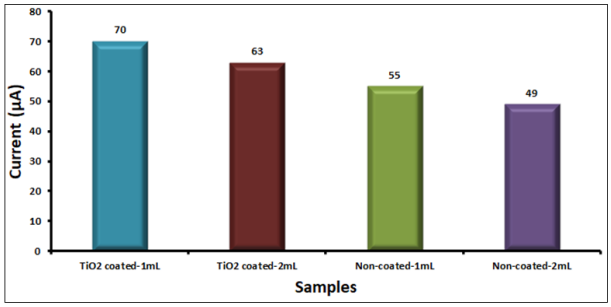

Figure 6: Bar graph for current difference with DPV studies

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REVIEW ARTICLE

A Systematic Review on Hepatocellular Carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is a cancer that accounts for a significant number of cancer-related mortality around the world. Curative therapy, such as surgical resection, liver transplantation, and local ablation, can improve a patient's chance of survival if they are administered early on. However, the disease is discovered when it is in an advanced state, and some of the available treatments are limited to palliative care and localized treatment only. Early identification of HCC, as well as appropriate treatment, are critical for increasing patient survival while also improving the patient's quality of life. Several aspects of hepatocellular carcinoma are discussed in this review, including the epidemiology, major risk factors, and therapeutic options.

Keywords: Hepatocellular carcinoma, Epidemiology, Diagnosis, Treatment.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most generally recognized vital liver disease, and it is also the leading cause of cancer-related death. Hepatocellular carcinoma accounts for more than 5 percent of all cancers in the globe, and the estimated number of cancer growth-related deaths exceeds 500,000 per year [1] In Africa and Southern Asia, the role of diethylnitrosamine, aflatoxin B1, and HBV infection, which can be acquired upon entering the world or acquired from the get-go throughout everyday life, is overwhelmingly dominant. HCC occurs in these patients at a young age and without the presence of cirrhosis in the majority of them. In contrast, in Japan, Egypt, and Southern Europe, HCV is the primary driver of HCC, which occurs in people who are more advanced in their disease, with nearly all of them having cutting-edge fibrosis or cirrhosis as a result. Cirrhosis is primarily caused by HCV infection and alcohol consumption in Northern and Central European countries. Alcohol is still the most common cause of cirrhosis in France, accounting for 60 percent of all liver cancer occurrences over the most recent decade [2].

Malignant growth is the most widely recognized fundamental liver hazard, and it is the leading source of malignant growth-related passing throughout the world. Health care costs are the eighth most common reason for cancer-related death in the United States [3]. An estimated total of 30,640 new liver and intrahepatic bile duct illnesses were predicted to occur in 2013, with a total of 21,670 deaths reported [4]. Males were more likely than females to develop HCC (2.4:1), and the disease was more common in Eastern and Southern Asia, Middle and Western Africa, Melanesia, and Micronesia/Polynesia than anywhere else in the world [5]. In the United States, the age-changed incidence of liver disease progression has increased from 1.6 per 100,000 people to 4.6 per 100,000 people among American Indians and Alaska Natives. This was followed by blacks, whites, and Hispanics [6].

Epidemiology

According to the World Health Organization, high-grade cervical cancer (HCC) is currently the fifth most common disease in the world and the third leading cause of cancer-related mortality. There were around 782,000 cases worldwide in 2012, with 83 percent of those infections occurring in less developed regions of the world, according to the estimates. In eastern Asia and sub-Saharan Africa, the annual occurrence rate exceeds 15 per 100,000 people, whereas figures are middle of the road (between 5 and 15 per 100,000 people) in the Mediterranean bowl and southern Europe, as well as North America, and low (under 5 per 100,000 people) in northern Europe [7]. A total of 39,230 new cases of HCC were projected in the United States in 2016, with 27,170 new HCC deaths, according to the National Cancer Institute's Surveillance Epidemiology End Result (SEER) program. Hepatitis B virus (HBV) vaccination has resulted in a reduction of the incidence of hepatocellular carcinoma (HCC) in countries where the virus was previously extremely prevalent [8]. These findings suggest that geographic variability is mostly associated with differences in the presentation rate to random events and the season of securing, rather than with differences in hereditary predilection to a certain location. A significant frequency of HCC has been found in temporary populations that is available in their local nations, although the occurrence of HCC reduces as the population matures [9]. Depending on factors such as sexual orientation, regional location, and a risk factor associated with malignant development improvement, the age at which HCC manifests itself is different. It is usual for the mean age at analysis to be less than 60 years in high-hazard countries with high HBV prevalence [10]. Cases tend to manifest themselves after the age of 60 in middle or low occurrence zones. African and Asian countries have attributed the analysis of HCC at earlier ages to a cooperative energy between HBV and dietary aflatoxins, which is assumed to be responsible for ctuate modifications in the TP53 quality [11]. The incidence of HCC has been steadily increasing in the United States over the last two decades [12].

Etiology

It is believed that cirrhosis, which is characterized by fibrosis associated with nodular healing, is a premalignant disease [13]. The majority of HCC patients in Western nations, including Brazil, are detected with cirrhosis helper to determined illness associated with either hepatitis B or C infections [14]. Furthermore, alcohol has been shown to be a substantial risk factor for cirrhosis and HCC development. Patients with non-alcoholic steatohepatitis (NASH) are at increased risk for liver cirrhosis and HCC, particularly if they are physically fit. Aflatoxin and metabolic diseases, such as hemochromatosis, type I glycogenosis, alpha-1-antitrypsin deficiency, Wilson's disease, and porphyrias, are other potential dangers at the beginning of a race. HCC can emerge in the absence of obvious hazard factors only in rare instances. for example, the fibrolamellar type is routinely irregular in the presence of previous cirrhosis or viral liver illness [15]. Viral illness, whether HBV or HCV, is associated with a significant risk of developing HCC, with cirrhotic individuals infected with either virus experiencing a 3–5 percent annual risk of developing HCC [16,17]. Different factors, such as insertional mutagenesis and family ancestry, could have a role in the progression of HCC at an earlier age. Overall, males outnumber girls in all zones, with the sex proportion often falling between 2:1 and 4:1. In many zones, females are younger than males at the time of analysis, which is a result of a genetic difference. Hormones associated with sexual activity appear to be a risk factor for HCC development. As a positive regulator of hepatocyte cell cycle regulators, testosterone speeds up the process of hepato-carcinogenesis; on the other hand, estrogen inhibits cell cycle regulators, suffocating the progression of liver malignant development [18].

Pathophysiology

Cirrhotic livers with chronic inflammation and fibro-genesis are the most typical sites of HCC. Inflammation and fibro-genesis increase the likelihood of liver dysplasia, which can progress to cancer. Provocative microenvironment plays a significant role in the initiation of the progression towards HCC. HCC is generated by a range of genetic/epigenetic aberrations and changes that affect a variety of signalling pathways, resulting in a known heterogeneity in the disease's biologic and clinical conduct. Although hepatectomies account for the majority of specimens, they represent only a small proportion of all patients. Hepatocellular carcinoma (HCC) exhibits extraordinary genetic diversity. There are differences across patients, including variances among stages of tumor development in a similar for a comparative patient, for example, in the knobs, as well as variation inside a tumour[19]. Recent research has been conducted to determine the genetic pathways that are impacted during the process of hepatocarcinogenesis [20]. Patients' p53, PIK3CA, and -catenin genes appear to be often mutated, according to the research. The identification of the signal pathways that are disrupted, resulting in uncontrolled division, will require additional research. Two cell separation pathways (i.e., WNT-catenin and Hedgehog) appear to have been tenfold altered. Pre-neoplastic adenomas with more pronounced potential for malignant transformation [21,22] are believed to benefit from up-directed WNT flagging.

Manifestations

Hepatocellular carcinoma is a cancer of the liver that often does not manifest itself until the later stages of the disease. The following are some of the most common hepatocellular carcinoma symptoms: Weight loss (without trying), loss of appetite, feeling very full after a small meal, fatigue, nausea, and vomiting are all symptoms of an enlarged liver, as is abdominal (belly) pain or pain near the right shoulder blade. Swelling or a build-up of fluid in the abdomen is also a symptom of enlarged liver (belly), Itching and yellowing of the skin and eyes (jaundice) [23,24,25].

Diagnosis

Hepatocellular carcinoma is diagnosed using a variety of tests and procedures, including blood tests to monitor liver function, imaging tests such as CT and MRI scans, and liver biopsy. AFP is a serum glycoprotein that was first identified as a marker for HCC and has since been shown to detect preclinical HCC [26]. Tissue des-gamma-carboxy prothombin (DCP), also known as PIVKA II (protein caused by vitamin K deficiency), is a frequently utilized tumor marker with a good specificity for colorectal cancer (HCC) [27].The use of imaging in the diagnosis of HCC is quite important. Ultrasound (US) imaging is frequently used in conjunction with, or I n lieu of, AFP imaging to aid in the detection of tiny hepatic tumours less than 3 cm in size. [28]. As a result of its limited sensitivity and positive predictive value in the presence of concomitant cirrhosis, it has generally been superseded as the diagnostic instrument of choice by CT scan and magnetic resonance imaging (MRI). Intratumoral fibrous stranding (mosaic sign), fatty transformation, necrosis, and calcification have all been observed on CT scans of individuals with probable HCC [29,30].

Treatment

Treatments for hepatocellular carcinoma include the following

Surgery. Early-stage liver cancer patients with normal liver function who undergo surgery to remove the cancer and a margin of good tissue surrounding it may have a better chance of survival than those with advanced liver cancer. Liver transplantation surgery is an option. Surgery to remove the entire liver and replace it with a liver from a donor may be an option for persons with liver cancer who are otherwise healthy and whose cancer has not spread beyond the liver. Using heat or ice to kill cancer cells is an option. For those who are unable to undergo surgery, ablation methods that use high heat or cold to eliminate cancer cells in the liver may be indicated. Radiofrequency ablation, cryoablation, and ablation with alcohol or microwaves are some of the techniques that are available. Direct delivery of chemotherapy or radiation to cancer cells is possible. Doctors can deliver chemotherapy medications (chemoembolization) or tiny glass spheres holding radiation (radioembolization) directly to cancer cells with the

help of a catheter that is pushed through blood arteries and into liver. Radiation therapy is another option. If surgery is not an option, radiation therapy, which uses X-rays or protons to deliver energy to the tumor, may be advised. Sophisticated radiation therapy, referred to as stereotactic body radiotherapy (SBRT), is used to treat cancer patients by directing many beams of radiation concurrently at a single place on their bodies. Drug therapy that is specific to the patient's needs. Cancer cells are targeted by targeted medications, which attack specific flaws in the cancer cells. These drugs may be able to delay the growth of the disease in persons who have advanced liver cancer. Immunotherapy. Immunotherapy medications work by activating your body's natural germ-fighting immune system to attack cancer cells in the body. When it comes to treating advanced liver cancer, immunotherapy may be an option.

CONCLUSION

In conclusion, the development of HCC is the consequence of a complex process that includes cirrhosis, HBV and HCV infection, alcohol intake, exposure to fungal aflatoxins, nicotine, and hereditary factors among other things. The illness stage determines which curative and/or palliative treatments are available. These treatments have a high cure rate and a low relapse rate, thus they are frequently utilized and have favourable outcomes. All treatments, however, have the potential to have side effects that negatively impact the patients' quality of life. The search for new molecular biomarkers with improved sensitivity and reliability has led to the discovery of the GP73, GPC3, OPN, and micro RNAs among other candidates. Micro RNAs have the ability to regulate a variety of biological pathways, many of which are directly associated to tumour growth and angiogenesis. This has the potential to improve the diagnosis, prognosis, and treatment of HCC. Having a greater understanding of these indicators makes it easier to diagnose HCC, which will result in a considerable benefit to patients through the development of new therapeutic targets and the subsequent increase in the cure rate. As a result, additional research is required to completely understand the process of hepatic carcinogenesis.

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REFERENCES

1. Bosch FX, Ribes J, Borras J. Epidemiology of primary liver cancer. Semin Liver Dis 1999;19:271–285.
2. Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. J Hepatol 1999;31:133–141.
3. Center for Disease Control and Prevention (CDC). Hepatocellular carcinoma-United States 2001–2006. MMWR Morb Mortal Wkly Rep. 2010; 59(17):517–520.
4. Crissien AM, Frenette C. Current Management of hepatocellular carcinoma. Gastroenterol Hepatol. 2014;10(3):153–161.
5. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893–2971.
6. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality and survival trends in the United States from 1975 to 2005. J Clin Oncol. 2009;27:1485–1491.
7. Ferlay J SI, Dikshit R, et al. Cancer incidence and mortality: sources, methods and major patters in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-E86. doi: 10.1002/ijc.29210.
8. Chiang CJ YY, You SL, Lai MS, Chen CJ. Thirty-year outcomes of the national hepatitis B immunization program in Taiwan. JAMA. 2013;310(9):974-6. doi: 10.1001/jama.2013.276701.
9. McCredie M, Williams S, Coates M. Cancer mortality in East and Southeast Asian migrants to New South Wales, Australia, 1975–1995. Br J Cancer. 1999;79(7-8):1277-82. doi: 10.1038/sj.bjc.6690205.

10. Bosch FX, Ribes J, Díaz M, Cléries R. Primary liver cancer: Worldwide incidence and trends. Gastroenterology. 2004;127(5):S5-S16. doi: 10.1053/j.gastro.2004.09.011.
11. Chan AJ BD, Kikuchi L, et al. Early Age Hepatocellular Carcinoma Associated with Hepatitis B Infection in South America. Clin Gastroenterol Hepatol. 2017;15(10):1631-2. doi: 10.1016/j.cgh.2017.05.015.
12. Kumar V, Abbas AK, Aster JC, editors. Robbins &Contran. Pathologic Basis of Diseases. 9th ed. Philadelphia: Elsevier; 2014.
13. Carrilho FJ, Kikuchi L, Branco F, Goncalves CS, Mattos AA; Brazilian HCC Study Group. Clinical and epidemiological aspects of hepatocellular carcinoma in Brazil. Clinics (Sao Paulo). 2010;65:1295-90.
14. Huguet C, Stipa F, Gavelli A. Primary hepatocelular cancer: western experience. In: Blumgart LH, editor. Surgery of the Liver and Biliary Tract. New York: Churchill Livingstone; 1996. Pp. 1365-1369.
15. Sakuma K, Saito N, Kasai M, et al. Relative risks of death due to liver disease among Japanese male adults having various statuses for hepatitis B s and e antigen/antibody in serum:a prospective study. Hepatology 1988;8:1642–6.
16. Tsukuma H, Hiyami T, Tanaka S, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797–801.
17. Tagger A, Donato F, Ribero ML, et al. Case-control study on hepatitis C virus (HCV) as a risk factor for hepatocellular carcinoma: the role of HCV genotypes and the synergism with hepatitis B virus and alcohol. Brescia HCC Study. Int J Cancer 1999;81:695–9.
18. Petrick JL BM, Laversanne M, Valery PC, Bray F, McGlynn KA. International trends in liver cancer incidence, overall and by histologic subtype, 1978–2007. Int J Cancer. 2016;139(7):1534-45. doi: 10.1002/ijc.30211.
19. Pok S BV, Wong HJ, et al. Testosterone regulation of cyclin E kinase: A key factor in determining gender differences in hepatocarcinogenesis. J Gastroenterol Hepatol. 2016;31(6):1210-9. doi: 10.1111/jgh.13232.
20. Shiratori Y, Shiina S, Imamura M. Characteristic difference of hepatocellular carcinoma between hepatitis B and C-viral infection in Japan. Hepatology 1995;22:1027–33.
21. Faivre J, Forman D, Esteve J, et al. Survival of patients with primary liver cancer, pancreatic cancer and biliary tract cancer in Europe. EUROCARE Working Group. Eur J Cancer 1998;34:1103-9.
22. Yang Q, McDonnell SM, Khoury MJ, et al. Hemochromatosis-associated mortality in the United States from 1979 to 1992: an analysis of Multiple-Cause Mortality Data. Ann Intern Med 1998;129:946–53.
23. Abou-Alfa GK, Jarnigan W, Dika IE, D'Angelica M, Lowery M, Brown K, et al. Ch. 77 - Liver and Bile Duct Cancer. In: Niederhuber JE, Armitage JO, Doroshow JH, Kastan MB, Tepper JE, eds. Abeloff's Clinical Oncology. 6th ed. Philadelphia, Pa: Elsevier; 2020:1314–1341.
24. National Cancer Institute. Physician Data Query (PDQ). Adult Primary Liver Cancer Symptoms, Tests, Prognosis, and Stages – Patient Version. Accessed at https://www.cancer.gov/types/liver/patient/about-adult-liver-cancer-pdq on March 12, 2019.
25. Schwartz JM, Carithers RL, and Sirlin CB. Clinical features and diagnosis of hepatocellular carcinoma. UpToDate website.https://www.uptodate.com/contents/clinical-features-and-diagnosis-of-hepatocellularcarcinoma.Updated November 2, 2018. Accessed March 12, 2019.
26. Kashyap R, Join A, Nalesnik M, Carr B, Barnes J, Vargas HE, et al. Clinical significance of elevated alpha-fetoprotein in adults and children. *Dig Dis Sci.* 2001;46: 1709–13.
27. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med.* 1984;310:1427–31.
28. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology.* 1995;22:432–8.
29. Stevens WR, Gulino SP, Batts KP, Stephens DH, Johnson CD. Mosaic pattern of hepatocellular carcinoma: histologic basis for a characteristic CT appearance. *J Comput Assist Tomogr.* 1996;20:337-42.
30. Stevens WR, Johnson CD, Stephens DH, Batts KP. CT findings in hepatocellular carcinoma: correlation of tumor characteristics with causative factors, tumor size, and histologic tumor grade. *Radiology.* 1994;191:531–7.

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RESEARCH ARTICLE

Nutritional, Sugar and Chemical Compound Profiling of Palmyra Sap (*Borassus flabellifer* L.)

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ABSTRACT

Palm sap is high in nutrients and finds application in the various food industries, many in the replacement of commercial sugar. The present study aimed to analyse nutritional, sugar and chemical compound profiling. For this study palmyra sap collected from Neelamedu, Tiruchegode Taluk, Namakkal District, Tamilnadu. The result of the proximate analysis showed1.42 g/100 ml of protein; 14.0 g/100 ml of total sugar; 15.2 g/100ml of TSS; 6.7 pH; 86.31 Moisture; 0.35g of total ash; 13.22 g of carbohydrate; 53.36 kcal of energy and 23.4 mg/100 ml of Vit C were present in Palmyra sap Antioxidant activity was tested using ferric reducing and ABTs+ assay and result showed that Palmyra sap showed promising antioxidant activity. Sugar profiling utilising the high-performance liquid chromatography revealed the presents of fructose and sucrose and GC/MS analysis was also tested. This paper provides complete data about the nutritional properties of Palmyra sap and this information can be explored further to enhance the industrial value of the Palmyra sap.

Keywords: Palmyra, Sap, Proximate analysis, Sugar profiling, Compound characterization

INTRODUCTION

Palm plants, which belongs to the family Arecaceae are generally perennial flowering plants and they are tree-like and stemless plants. Nearly 181 genera and 2,600 species were reported in tropic and subtropic regions (*Christenhusz & Byng, 2016*). Palm trees have numerous additional benefits, such as being extremely efficient at turning solar energy into biomass in most tropical agro-ecological zones. Throughout the tropical world, palm species have been used for the production of various food products such as juice, fermented drinks, syrup, refined and brown sugar. Generally, palm trees have been tapped because they produce sap which is rich in sugar and these have been exploited for various applications. The benefits of extracting sugar from sap before it reaches the fruits are obvious. These sugars are intercepted before being used in the production of the non-edible parts like the husk in coconut and the production of edible material. As a result, tapping a palm for the sap rather than letting the palm to grow fruits is

more economical in terms of edible energy production. Similarly, it was proven that, in the context of harvestable energy from the coconut palm. The harvested energy from the sap could be 5 to 7 times higher than from the oil of the nuts (Banzon, 1984). In another study, (Apriyantono *et al*., 2002) proved that coconut and palm sugar contains major sugars like glucose, fructose and sucrose at the amount of 89 to 91%. During heating, due to presences of sugar, palm sap may undergo maillard reaction and caramelization. Pyrazines, furans, ketones, fatty acids, and organic acids constitute volatile components of coconut and palm sugar, and they are responsible for their sweet, toasty, and nutty caramel-like aroma (Sohn and Ho 1995).The state tree of Tamil Nadu is Borassus flabellifer L., and its palm sugar was consumed in India in the fourth century BC. In addition, they provide many medicinal, economic ecological, and sociological advantages to society. These plants can withstand various adverse climatic conditions and natural calamities. From India through south-east asiato New Guinea, these plants widely found in tropical and dry regions. The plant has a very close connection with the rural livelihoods, as well as cottage and agro-based enterprises of the Indian economy. This plant's usage classified into three categories such as non-edible, edible, and value-added. Palm sap has a significant economic value which used in various forms like drinks, alcoholic beverages, vinegar and acetic acid etc (Ghosh *et al*., 2018).Barh and Mazumdar (2008) found that palm sap is the richest source of nutrients compared with those of sugar palm juice and date palm sap. In this context, the present study attempted to study the nutritional analysis, antioxidant activity and chemical compound profiling using HPLC of palmyra sap.

MATERIALS AND METHODS

Collection of Sample

Palmyra sap was freshly collected from Neelamedu, Tiruchegode Taluk, Namakkal District, Tamilnadu and all samples were stored in an icebox and immediately transported to the Laboratory. The sap was filtered by using the cotton muslin cloth and stored in a refrigerator for further analysis.

Proximate Composition

Thee proximate analysis was performed using an AOAC standard technique for the study of moisture, ash, and crude fat content (AOAC, 2000) and the micro-Kjeldahl technique was used to determine the crude protein(AOAC, 2012).Milwaukee pH-600was used to determine the pH of palm sap at room temperature(25±1°C) and total soluble solids (TSS) was measured using the ATC-Hand held Brix Refractometer (RHB-90ATC) with a wide TSS measuring range (0%–90%).

Antioxidant Activity

ABTS(2,2'-Azino-Bis(3-Ethylbenzothiazoline-6-Sulphonic Acid)Radical Scavenging Assay

The study material was subjected to an ABTS radical scavenging test using modified technique of Perumal *et al.* (2018). The ABTS (7 mM, 25 ml in deionized water) stock solution was prepared with potassium persulfate ($K_2S_2O_8$) (140 mM, 440 µl). Different concentrations of test samples and standard (Ascorbic acid) were mixed with the ABTS working solution (2.0 ml) and the reaction mixture was allowed to stand at room temperature for 20 min; then, the Abs was measured using an ultraviolet-visible spectrophotometer at 734 nm. The radical scavenging activity was given as ABTS radical scavenging effect was calculated by the equation:

ABTS radical scavenging effect (%) = [(A0 - A1)/A0] ×100

Where A0 is the control; A1 is the test

Ferric Reducing Antioxidant Potential Assay

The antioxidant capacity of the given samples was determined by spectrophotometrically following the modified procedure of Benzie and Strain (1999). The method is based on the reduction of Fe^{3+} TPTZ complex (colourless complex) to Fe^{2+}-tripyridyltriazine (blue coloured complex) formed by the action of electron-donating antioxidants at low pH. This reaction is observed by measuring the change in absorbance at 593 nm. The Ferric reducing antioxidant

power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°C. 2 ml of freshly prepared working FRAP reagent was pipetted and mixed with various concentrations of the standard (5, 10, 20, 40, 80,160 and 320 µg/ml) and test samples (5, 10, 20, 40, 80,160 and 320 µg/ml) were mixed thoroughly. An intense blue colour complex was formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form and the absorbance at 593 nm was recorded against a reagent blank after 30 min incubation at 37°C.

Sugar Profiling

Sugar profiling was performed using high-performance liquid chromatography (HPLC) comprised of a Waters Alliance 2695 separation module (Waters Corporation) equipped with a refractive index detector (RID; Waters 2414 Corporation). Obtained Sap from the coconut tree was diluted ten times with deionised water and then filtered using a 0.45 µm nylon filter, 20 µl of the sample were the mobile phase consisted of HPLC-grade acetonitrile and double-distilled water (80:20, v/v ratio; Chang, Karim, Mohammed, & Ghazali, 2018). The sugars were separated isocratically at a flow rate of 1.5 ml/min. Standard curves were constructed based on sugar reference standards (fructose, glucose, and sucrose) by plotting peak area against various concentrations of each sugar (Chang *et al*., 2018)and water (80:20, v/v ratio).

Compound Profiling

GC-MS analysis of the palmyra sap was carried outby following the method of Kumaravel *et al*. (2010)GC/MS analysis was performed using a PerkinElmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1,fused silica capillary column (30 m x 0.25mmIDx 0.25 µdf, composed of 95% Dimethyl polysiloxane). Helium gas was used as the carrier as at a constant flow rate of 1 ml/min. and an injection volume of 2 µl was employed (splitratio of 10:1). Injector temperature 250°C; Ion source temperature 280°C. The oven temperature was programmed from 110°C(isothermal for 2 min.), with an increase of10°C/min, to 200°C, then 5°C/min to 280°C,ending with a 9 min. isothermal at 280°C. Mass spectra of compounds in the sample obtained by electron ionization (EI) at 70 eV; a scan-interval of 0.5 seconds and fragments from 45to 450 Da. Total GC running time was 36 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatograms was a Turbomass.

RESULTS AND DISCUSSION

Proximate composition varies from plant to plant and it mainly depends on the type of food, the species, the origin, the climate conditions in which the plant was raised and many other factors. In the present study, the proximate composition of palm sap tabulated in Table. 1 and the result showed that 1.42 g/100 ml of protein; 14.0 g/100 ml of total sugar; 15.2 g/100ml of TSS; 6.7 pH; 86.31 Moisture; 0.35g of total ash; 13.22 g of carbohydrate; 53.36 kcal of energy and 23.4 mg/100 ml of Vit C were present in Palmyra sap. The present result was correlated with previous finds which includes the proximate composition of African Palmyra palm was tested by Umar *et al*. (2017) and the result showed 56.33% w/w moisture, 11.2%DW crude fiber, 6.9%DW crude protein and 8.1%DW available carbohydrate. In another study, Oryema and Oryem-Origa (2016) a proximate analysis for Palmyra pulps using a wet matter (WM) basis and dry matter (DM). Djibrilla (2006) studied the proximate composition of flour from *B. aethiopum* fruit pulp and the study recorded 91.79%; total ash content of 2.7%; total lipid content of 0.16%; crude protein of 4.23%; crude fibre of 29.75%. Proximate composition together with the mineral composition of a particular product is termed the nutritional composition of that particular product. Antioxidants have been tested with various sources and many effective methods have been employed to determine the antioxidant activity in food and plant sources (Antolovich *et al.,* 2001). Several methods were proven to be efficient in determining the antioxidants activity plant source which includes the Peroxide Value test, Anisidine Value, ABTS+ assay and DPPH free radicals assay. In the present study, ferric reducing and ABTs+ assay was employed to study the antioxidant activity of Palmyra sap (Fig. 1 & 2). In the result of the ferric reducing assay, the increased concentration of Palmyra sap showed increased

ferric reducing activity (0.115) at 320 µg of sap tested (Fig. 3). In ABTs assay, the IC_{50} value of the given sample was found (>320 µg/ml) and the standard drug (Ascorbic acid) was 14.56 µg/ml, respectively (Fig. 4).$ABTS^{*+}$ produce more powerful free radicals than $DPPH^{*}$ radicals and the reactions with $ABTS^{*+}$ radicals involve a single electron transfer process (Davis & Johnson, 1987, Asghar *et al*., 2019). The principle of $ABTS^{*+}$ assay is that the preformed radical monocation of $ABTS^{*+}$ is generated by oxidation of $ABTS^{*+}$ with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants.

The HPLC profiles of sugar in Palmyra sap are shown in Fig. 2 and the result elaborated the presents of two sugars which includes sucrose and fructose (Fig. 5). Totally 89.87% of sugar was recorded of which 0.75% detected as fructose and 89.12% as sugar content. As a conclusion this sugar profiling, it is proven that sucrose content was higher when compared to fructose. This result was correlated with the previous finding of Somawiharja *et al*. (2018), the result states that the amounts of sucrose, fructose, and glucose in fresh palm sap were 1.76%, 5.76% and 4.46%, respectively. On the other hand, the recorded values of sugar palm juice exhibited higher fructose (1.46%) and glucose (1.08%) contents but lower sucrose content (10.88%) compared with fructose (ND), glucose (ND), and sucrose (13.81%) as reported by Veena *et al*. (2018). Among the carbohydrate, sucrose causes a less fattening effect than glucose or starch (Jentjens and Jeukendrup 2005). Ruzzin *et al*. (2005) also reported a 15% increase in energy consumption by sucrose without an increase in weight gain. The compound present in the Palmyra sap was tested using GC-MS and the result showed the presence of compound which represented in the form of major and minor peaks. The GC- MS chromatogram of the ten peaks of the compounds detected are shown in figure 6. The major and minor compounds with their retention time (RT), molecular formulae, molecular weight (MW) and peak area (%) are presented in Table 2. Thus, the present study can be concluded that the Palmyra sap may be considered as a rich source of nutrients and also a candidate with great antioxidant activity. The study also concluded that sugar present in the Palmyra sap can be the alternate source of other sugars available in the market, for commercialization more research is needed.

REFERENCES

1. Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S. and Robards, K. (2001). Methods for Testing Antioxidant Activity. The Analyst, 127:183-198.
2. AOAC (2000). Official methods of analysis (17th ed.). Gaithersberg, MD: Association of Official Analytical Chemists.
3. AOAC (2012). Official methods of analysis of AOAC International. Gaithersberg, MD: AOAC International
4. Apriyantono, A., A. Aristyani, Y. Nurhayati, S.Budiyanto Lidya, and S.T. Soekarto. 2002. Rate of browning reaction during preparation of coconut and palm sugar. International Congress Series 1245: 275–278.
5. Asghar, Muhammad & Yusof, Yus & Mokhtar, Mohd & Ya'acob, M. & Mohd Ghazali, Hasanah & Chang, Lee Sin & Manaf, Yanty. (2019). Coconut (*Cocos nucifera* L.) sap as a potential source of sugar: Antioxidant and nutritional properties. Food Science & Nutrition. 8. 10.1002/fsn3.1191.
6. Banzon, J.A. 1984. Harvestable energy from the coconut palm. Energy in Agriculture, 3: 337-344.
7. Barh, D., & Mazumdar, B. (2008). Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date (*Phoenix sylvestris* Roxb.) sap in treatment of anemia. Research Journal of Medicine and Medical Sciences, 3, 173–176.
8. Benzie, I.F.F.; Strain, J.J. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 1999, 299, 15–27.
9. Chang, L. S., Karim, R., Mohammed, A. S., & Ghazali, H. M. (2018). Characterization of enzyme-liquefied soursop (*Annona muricata* L.) puree. LWT-Food Science and Technology, 94, 40–49

10. Christenhusz, M. J. M. & Byng, J. W. (2016). "The number of known plants species in the world and its annual increase". Phytotaxa. 261 (3): 201–217. doi:10.11646/phytotaxa.261.3.1. Archived from the original on 2016-07-29.
11. Davis, T. & Johnson, Dennis. (1987). Current utilization and further development of the palmyra palm (*Borassus flabellifer* L., Arecaceae) in Tamil Nadu State, India. Economic Botany. 41. 247-266. 10.1007/BF02858972.
12. Djibrilla, A. (2006), Influence of drying on some physicochemical properties of *Borassus aethiopum* Mart fruit pulp. Thesis, Master in Food Science and Nutrition, University of Ngaoundere, 61.
13. Ghosh, D., Bandyopadhyay, A., Das, S., Hebbar, K., & Biswas, B. (2018). Coconut sap (Neera)-untapped opportunity of spinoff gains in West Bengal, India. International Journal of Current Microbiology and Applied Sciences, 7, 1883–1897.
14. Jentjens, R.L., and A.E. Jeukendrup. 2005. High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. British Journal of Nutrition 93(4): 485–492.
15. Oryema, C. and Oryem-Origa, H. (2016). "Analysis of the Fresh Pulps of *Borassus aethiopum* Fruits of Gulu District, Uganda." American Journal of Food and Nutrition, 4(6): 177-181.
16. Perumal, p., and k. Saravanabhavan. "Antidiabetic and Antioxidant activities of ethanolic extract of *Piper betle* l. Leaves in catfish, *Clariasgariepinus*". Asian Journal of Pharmaceutical and Clinical Research, Vol. 11, no. 3, Mar. 2018, pp. 194-8, doi:10.22159/ajpcr.2018.v11i3.22393.
17. Ruzzin, J., Y. Lai, and J. Jensen. 2005. Consumption of carbohydrate solutions enhances energy intake without increased body weight and impaired insulin action in rat skeletal muscles. Fructose and sucrose solutions enhanced energy intake but did not increase body weight. Diabetes & Metabolism 31: 178–188.
18. Sohn, M., and C.T. Ho. 1995. Ammonia generation during thermal degradation of amino acids. Journal of Agricultural and Food Chemistry 43(12): 3001–3003.
19. Umar, K.J., Abdullahi, B.M, Muhammad, B., Muhammad, S. Hassan, L.G. and Sani, N.A. (2017). Nutritional and Antinutritional Profile of *Borassus aethiopum* Mart (African Palmyra Palm) Shoots. International Journal of Sciences: Basic and Applied Research (IJSBAR). 39-49.
20. Veena, K.S. & Sameena, M.T. & Padmakumari, A.K.P. & Srinivasa, G.T.K. & Nishanth, K.S. & Reshma, M.V.. (2018). Development and validation of HPLC method for determination of sugars in palm sap, palm syrup, sugarcane jaggery and palm jaggery. International Food Research Journal. 25. 649-654.

Table 1: Proximate analysis of Palmyra sap

Parameter	Content
Protein	1.42 g/100 ml
Total sugar	14.0 g/100 ml
TSS	15.2 g/100ml
pH	6.7
Moisture	86.31
Total Ash	0.35g
Carbohydrate	13.22 g
Energy	53.36 kcal
Vit C	23.4 mg/100 ml

Table 2: Compound identified from the Palmyra sap using GC MS

RT	Name	Area	Formula	Mass	Area%
3.83	2-Cyclopenten-1-one,3-hydroxy-2-methyl-	342229	C6H8O2	112.1	0.62
3.99	3-Piperidinol,1-ethyl-	1145881	C7H15NO	129.1	2.07
4.13	Propanoicacid,anhydride	1881971	C6H10O3	130.1	3.40
4.22	Thymine	1776050	C5H6N2O	126	3.20
4.93	trans-3-Methyl-2-n-propylthiophane	681431	C8H16S	144.1	1.23
5.18	Propanoicacid,anhydride	332507	C6H10O3	130.1	0.60

5.27	.alpha.-Methyl-D-mannopyranoside	152381	C7H14O6	194.1	0.27
5.37	Catechol	445967	C6H6O2	110	0.80
5.48	l-Alanine,N-methoxycarbonyl-,tridecylester	253427	C18H35NO	329.3	0.46
5.64	Valericacid,4-nitrophenylester	224525	C11H13NO	223.1	0.41
6.01	3-Thiopheneethanol	707107	C6H8OS	128	1.28
6.15	Cyclopentanone, 2-(1-methylpropyl)-	441461	C9H16O	140.1	0.80
6.31	5-Hydroxymethylfurfural	3844076	C6H6O3	126	6.93
6.50	Tricyclo[3.3.1.1(3,7)]decane-1-carboxamide, N-[2-(2-thienyl)ethyl]-	203153	C17H23NO	289.2	0.37
6.75	Eugenol	2660313	C10H12O2	164.1	4.80
7.12	1,4-Benzenediol,2-methyl-	254268	C7H8O2	124.1	0.46
7.23	Silane, [(1,1-dimethyl-2-propenyl)oxy]dimethyl-	196195	C7H16OSi	144.1	0.35
7.30	Aziridine, 2-methyl-2-(2,2,4,4-tetramethylpentyl)-	406185	C12H25N	183.2	0.73
7.81	(E)1-Allyl-2-methylcyclohexanol	2605385	C10H18O	154.1	4.70
7.93	d-Ribose, 2-deoxy-bis(thioheptyl)-dithioacetal	178533	C19H40O3S	380.2	0.32
8.29	(2-Methyl-but-3-enyl-2-oxy)-trimethyl-silane	656316	C8H18OSi	158.1	1.18
8.54	Borinicacid,diethyl-	19725323	C4H11BO	86.1	35.58
8.65	4-tert-Butylcyclohexylmethylethylphosphonate	275069	C13H27O3	262.2	0.50
8.85	Butanoicacid,2-methyl-,pentylester	344429	C10H20O2	172.1	0.62
8.93	.beta.-D-Glucopyranose, 1,6-anhydro-	530645	C6H10O5	162.1	0.96
9.18	Benzenaldehyde, 2-fluoro-5-hydroxy-	361672	C7H5FO2	140	0.65
9.36	Lactose	420479	C12H22O1	342.1	0.76
10.02	3-Deoxy-d-mannoiclactone	8450609	C6H10O5	162.1	15.24
10.48	Decanoicacid,3-hydroxy-,methylester	318668	C11H22O3	202.2	0.57
10.59	.alpha.,.beta.-Gluco-octonicacidlactone	481894	C8H14O8	238.1	0.87
10.84	2-Propenoicacid,2-methyl-,octylester	183261	C12H22O2	198.2	0.33

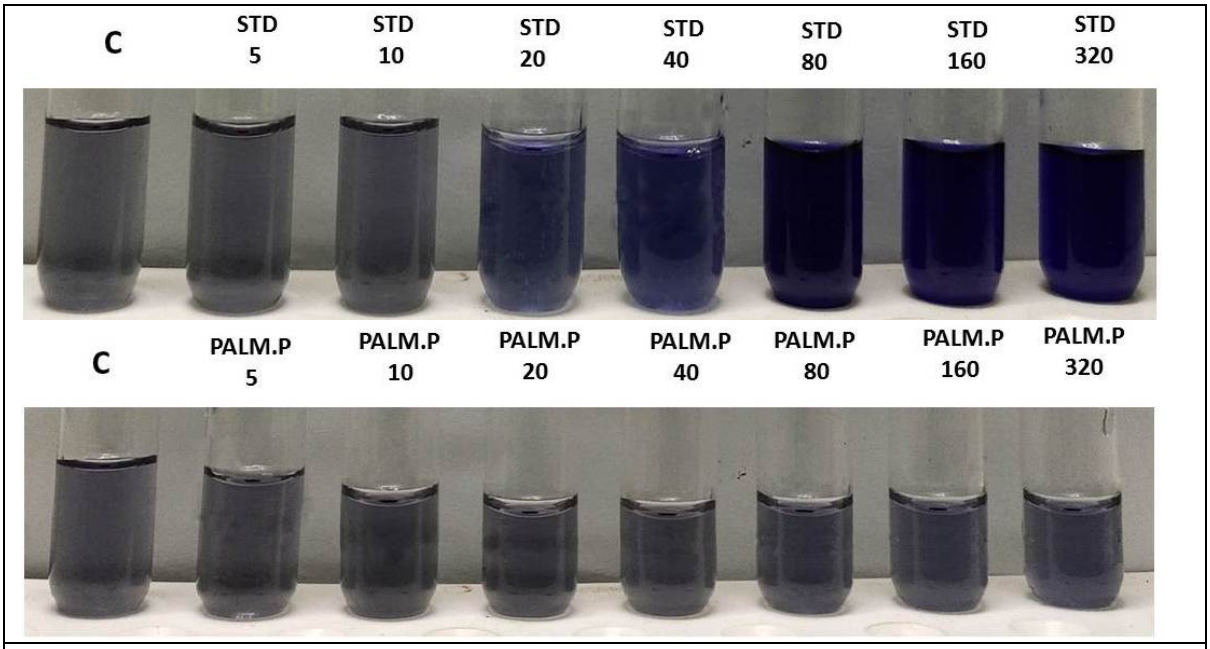

Fig 1: Ferric reducing assay for Palmyra sap against control (ascorbic acid)

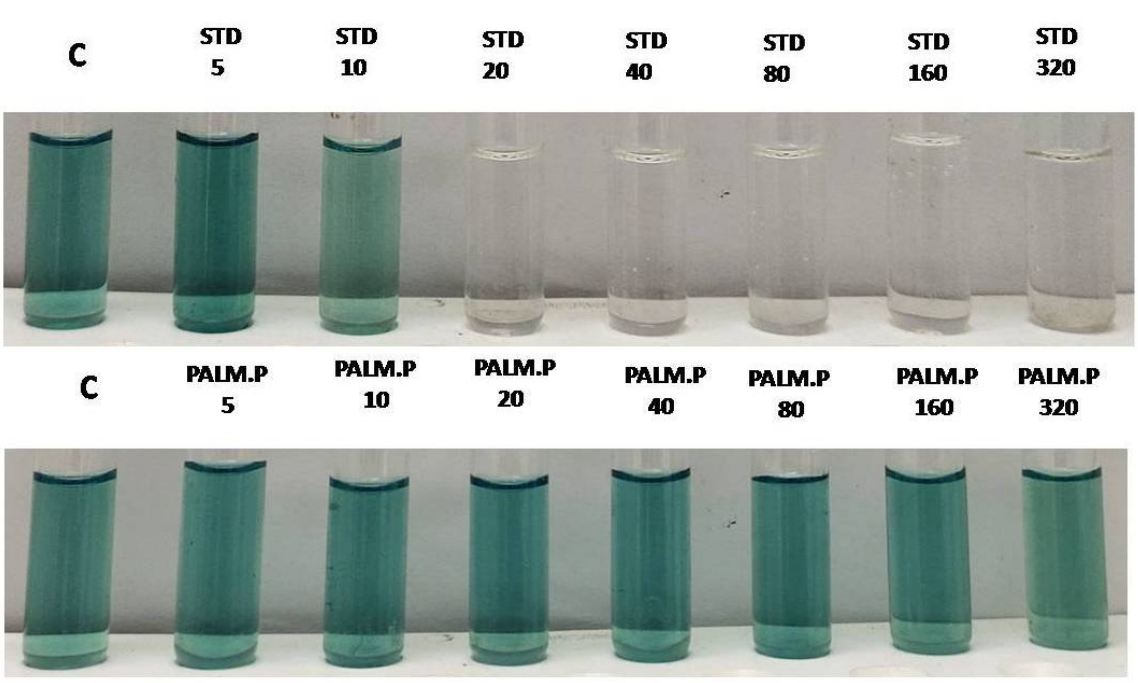

Fig 2: ABTs assay for Palmyra sap against control (ascorbic acid)

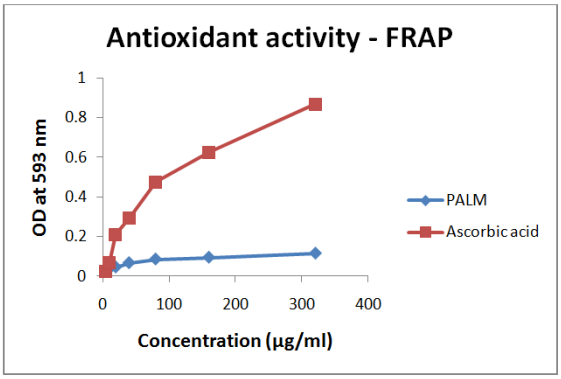

Fig 3: Antioxidant activity using ferric reducing assay

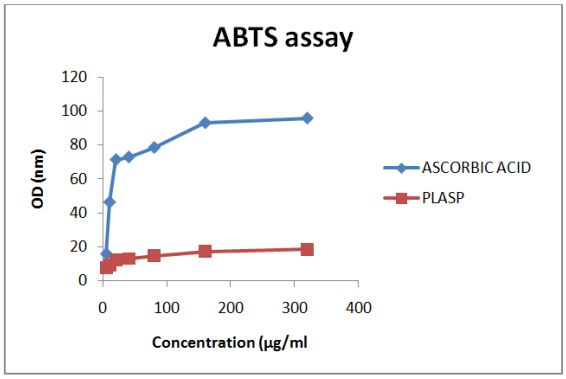

Fig 4: Antioxidant activity using ABTs assay

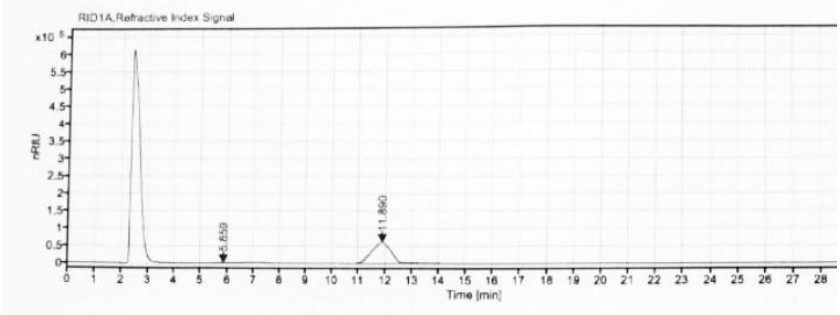

Fig 5: Sugar profiling of Palmyra sap using HPLC

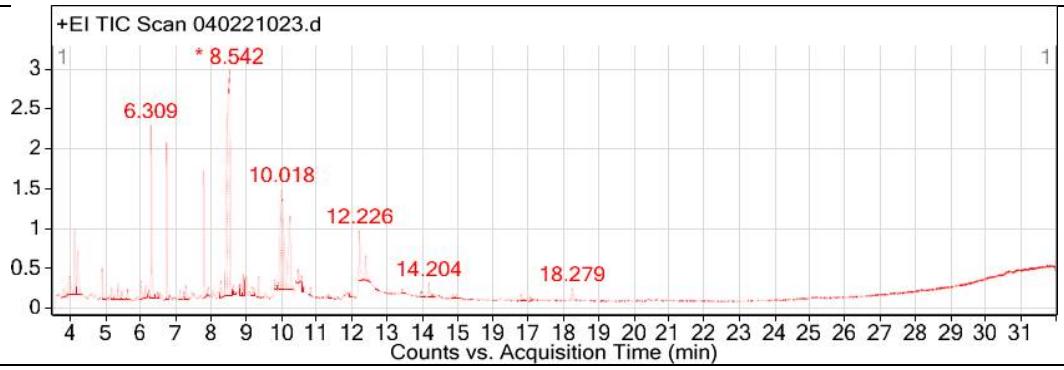

Fig 6: Compound profiling of Palmyra sap using GC MS

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RESEARCH ARTICLE

Effect of Eraippu NOI Chooranam (ENC) on Milk Induced Leukocytosis and Eosinophilia in the Management of Asthma

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ABSTRACT

Biologically active compounds from natural sources are of interest as they form the possible new drugs for many diseases. Eraippu Noi Chooranam is a modified Siddha Poly Herbal formulation indicated for respiratory diseases in the text Siddha research pharmacopoeia. The indicated traditional claim enforced to evaluate its efficacy in the management of Bronchial asthma. To evaluate the effect of Eraippu Noi Chooranam *(ENC)* in the management of Bronchial asthma. The medicine is prepared as per the method mentioned in the classic siddha literature. In the present study, aqueous extract of *ENC* at doses of 270,530,800 mg/kg p.o was evaluated for the management of Bronchial asthma using milk induced leukocytosis and eosinophilia in Swiss albino mice. The results of the present investigation showed that aqueous extract of *ENC* at doses of 270,530,800 mg/kg p.o significantly decreased milk induced leukocytosis and eosinophilia in mice in a dose dependent manner when compared with control group. It can be concluded that aqueous extract of *ENC* may be used in the management of asthma.

Keywords : Bronchial asthma, Siddha, Poly Herbal formulation, Eraippu Noi Chooranam

INTRODUCTION

Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness [1]. Medicinal plants based traditional systems of medicines are playing important role in providing health care to large section of population, especially in developing countries. It is a well-known fact that Traditional Systems of medicines always played important role in meeting the global health care needs. They are continuing to do so at present and shall play major role in future also. The system of medicines which are considered to be Indian in origin or the systems of medicine, which have come to India from outside and got assimilated in to Indian culture are known as Indian Systems of Medicine [2]. Indian Systems of Medicine are among the well known global traditional system of medicine.

Asthma is a chronic inflammatory disorder of respiratory system, which is characterized by narrowing of airways hyper-responsiveness and changes in the levels of mast cells, cytokines, lymphocytes and other related inflammatory cell products [3]. Currently, wide ranges of anti-asthmatic drugs are available for the treatment of asthma. However, these all are limited to short symptomatic relief and causes complex side effects. These adverse effects of synthetic drugs prompt a switch over to traditional complementary and alternative medicine [4]. Though Siddha drugs are considered to be safe and effective, it is the utmost duty of the physicians to validate the formulation before trying out in humans. Eraippu Noi Chooranam is a modified Siddha Poly Herbal formulation mentioned in the text Siddha research pharmacopoeia. It is indicated for asthma, chronic bronchitis and flatulence [5]. It is a poly herbal drug and all the ingredients included are very effective in curing kapha diseases. The indicated traditional claim enforced to evaluate its efficacy in the management of Bronchial asthma.

Aim and objectives

The aim of this study is to evaluate the anti asthmatic property of the drug Eraippu Noi Chooranam by Milk induced leucocytosis and eosinophilia.

MATERIALS AND METHODS

Collection and Identification of plant materials

The herbal ingredients were authenticated by the Assistant Professor of Medicinal botany, National Institute of Siddha, Chennai. The raw drugs were purified as per the methods mentioned in the literature.

Preparation of the drug Eraippu Noi Chooranam [5]

Ingredients

Kuppaimeni leaves choornam (*Acalypha indica*) - 224 gms
Chiru Cherupadai leaves choornam (*Mollugo lotoides*) - 224 gms
Potrilai kaiyan leaves choornam (*Eclipta prostrata*) - 224 gms
Vembu leaves choornam (*Azadiracta indica*) - 224 gms
Milagu fried chooranam (*Piper nigrum*) - 112 gms
Arisi thippili chooranam (*Piper longum*) - 112 gms
Amukkara chooranam (*Withania somnifera*) - 112 gms
Kadukkai thol chooranam (*Terminalia chebula*) - 112 gms
Cane sugar powder -392 gms

Purification of raw drugs [6,7]
The raw drugs are purified as per the methods mentioned in the Siddha literatures.

Preparation of trial drug
All the ingredients were powdered separately and mixed together as per the mentioned composition and bottled up.

Physicochemical Analysis [8]
Preliminary Physicochemical analysis of the test drug was carried out in the aqueous extract of ENC which revealed that it was of standard quality. Phytochemical analysis revealed the presence of phytosterols, flavanoides, aminoacids, carbohydrates, terpenoids, phenolic compounds and tannin.

Toxicity Study of ENC [9]
Single dose acute toxicity study revealed that Eraippu Noi Chooranam was safe and did not produce any toxic effect at the dose of 2000 mg/kg. Repeated dose administration of ENC in sub acute toxicity study reported that there were no treatment related histopathological abnormalities in any of the organs noticed and hence NOAEL of ENC was greater than 900 mg/kg/b.w in rats

Milk induced leucocytosis [10,11,12] (***In vivo* assessment of antistress (adaptogenic) activity)**
Animals
Swiss albino mice weighing between 25-35 gm were used for this study. The mice were purchased from Sree Venkateshwara Enterprises Pvt. Ltd, Bangalore and housed in standard laboratory condition in cages made of Polypropylene, in a well ventilated room under an environmental temperature of 22±3˚C and relative humidity of about 30-70%, with a 12-h light and 12 –h dark artificial light cycle and provided with food from 'Sai Durga Animal Feed, Bangalore and water *adlibitum.*

Mice were kept as five groups (n=5) and each group having 5 animals. Animals in group I was positive control and was administered with only boiled and cooled milk (4 ml/kg, s.c) Animals that are in group II served as standard and were given Dexamethasone (50 mg/kg i.p.) while animals that are in group III to V served as test group and received Eraippunoi Chooranam at a dose of 270,530,800 mg/kg p.o respectively prior to 1 hour of milk injection. The retro orbital plexus area was selected for collection of blood. Blood samples were collected using a glasscapillary under light anesthesia. Total leukocyte countwas done in all the group of animals before drug administration and 24hr after injection of milk. WBC pipette was used to suck blood up to mark and further diluted with WBC diluting fluid. Pipette was then shaken thoroughly for few seconds and kept aside for five minutes. The above fluid was charged in Neubaur's chamber and TLC was done. Difference in total leukocytes count before and 24 hr after administration of drug was calculated.

Milk induced eosinophilia(*In vivo* assessment of antiallergic activity)
Animals in group I was positive control and was administered with only boiled and cooled milk (4 ml/kg, s.c) Animals in group II served as standard and were given the standard drug Dexamethasone (50 mg/kg i.p.) while animals belonging to group III to V served as test group and received Eraippunoi Chooranam at a dose of 270,530,800 mg/kg p.o respectively1 hour before milk injection. Blood samples were taken from retro orbital plexus usinga glasscapillary under light anesthesia. Total eosinophil countwas done in all the groupof animals before drug administration and 24hr after injection of milk. Blood was sucked inWBC pipette to mark 1, which was followed by the eosin solution to mark 11. Mix the contents of the bulb by shaking it thoroughly for about 30-40 seconds and put it aside for 15-20 min for the purpose of lysis and staining. The above fluid was charged in Neubaur's chamber and eosinophil count was done.

RESULTS

Milk-induced leukocytosis in mice

The maximum increase in difference of leukocytes (4704.4± 144.5) was observed in control group 24 h after administration of milk (4 mL/kg, s.c).Animals treated with Dexamethasone (50 mg/kg, i.p.), has shown significant inhibition of milk-induced leucocytosis when compared to control ($p< 0.01$). Groups of mice pretreated with ENC at 270 mg/kg dose did not show any statistically significant inhibition in leukocytosis, while ENC at 530 mg/kg (2376.8± 199.2) and 800 mg/kg (2103.6±210.1) showed significant inhibition($p< 0.01$) of milk induced leucocytosis 24 h after treatment in dose dependent manner as shown in Table.1 and Fig.1 when compared with control group.

Milk-induced eosinophilia in mice

The maximum increase in difference of eosinophil (118.4± 2.34) count was observed in control group 24 h after administration of milk (4 mL/kg, s.c).Animals treated with Dexamethasone (50 mg/kg, i.p.), has shown significant inhibition of milk-induced eosinophilia as compared to control ($p< 0.01$). Groups of mice pretreated with ENC at 270 mg/kg (104.2±2.22),530 mg/kg (2376.8± 199.2) and 800 mg/kg (2103.6±210.1) showed significant inhibition($p< 0.01$) of milk induced eosinophilia 24 h after treatment in a dose dependent manner which is shown in Table.2 and fig.2 when compared with control group. Results are expressed as Mean± S.E.M. where n= 5Statistical analysis done by using ANOVA followed by Dunnett's test**$p<0.01$ when Group II, III, IV, V compared with Group I.

DISCUSSION

Milk induced Leucocytosis

Allergy and anaphylaxis are the most important factors responsible for diseases like rhinitis, cold, cough, asthma, bronchitis, pain, inflammation etc. The milk-induced leukocytosis and eosinophilia in mice model helps to evaluate the effect of test drug in stress-induced asthma. In the present investigation ENC at doses of (270,530,800 mg/kg, p.o.) was evaluated for management of asthma using milk induced leukocytosis and eosinophilia in mice. The various types of mediators released by leucocytes during inflammatory conditions of asthma are cytokines, histamine, and major basic protein, which promote the process of inflammation [13]. The infiltration of leukocytes potentiates the inflammatory process by releasing reactive oxygen species into the surrounding tissue, resulting in increased oxidative stress [14] and are associated with the pathogenic features of asthma [15]. In this study it was observed that decreased leukocytes count was noticed in mice treated with ENC at doses of 530,800 mg/kg significantly($p<0.01$) as compared to vehicle treated group. Result suggests that ENC decreases milk induced leukocytes count by normalizing oxidative stress.

Milk induced Eosinophilia

The Eosinophil are the most important characteristic inflammatory cells in bronchial biopsies taken from patients with asthma and may be seen in the epithelial and submucosal layers. An abnormal increase in the count of peripheral eosinophil to more than 4% of total leukocyte count is termed as eosinophilia. In patient with asthma there is an increase in eosinophil count and hypersecretion of mucus and airway hyper reactivity were stimulated [16,17] Eosinophils infiltrating in the airway also have tremendous effect on mucus secretion by epithelial goblet cells [18]. In bronchial mucosa, in which allergic inflammation occurs, due to the involvement of eosinophil is a critical contributor to the late asthmatic reaction of congestion of airway and mucus hypersecretion. Eosinophil plays role as inflammatory cell in the late phase, especially in the condition of allergic asthma. Eosinophil secretes mediators such as tumor necrosis factor, eosinophil-derived neurotoxin, eosinophil cationic protein, and prostaglandin, resulting in epithelial shedding, broncho constriction, and promotion of inflammation along the respiratory tract often allergic [19]. Immunomodulating agents are useful in treating allergy by virtue of inhibiting the antigen-antibody (AG: AB)

reaction thereby inhibits the release of inflammatory mediators [20]. The beneficial effect of *ENC* could be due to either inhibition AG: AB (hypersensitivity Reaction-I) i.e. by having antiallergic and anti-inflammatory properties.

CONCLUSION

From the results obtained in the present investigation it can be concluded that aqueous extract of of *ENC* possesses significant adaptogenic activity,and antiallergic activity suggestive of its potential in prophylaxis and management of asthma. Hence, further detailed study needs to be conducted to evaluate the clinical efficacy in the treatment of asthmatic patients.

Source of Support
Nil

Conflict of Interest
None declared.

REFERENCES

1. Anonymous, https://www.who.int › Health topics
2. Prasad LV. In: *Indian System of Medicine and Homoeopathy Traditional Medicine in Asia.* Chaudhury Ranjit Roy, Rafei Uton Muchatar., editors. New Delhi: WHO- Regional Office for South East Asia; 2002. pp. 283–286.
3. 3.Bousquet J, Bousquet PJ, Godard P and Daures JP (2005): The public health implications of asthma. Bull World Health Organ 83: 548-554.
4. Govindan S, Viswanathan S, Vijayasekaran V, et al. (1999): A pilot study on the clinical efficacy of Solanumxanthocarpum and Solanumtrilobatum in bronchial asthma. J Ethnopharmacol 66:205-10.
5. The Pharmacopoeia of siddha medicines- Dr.M.Shanmugavelu & Dr.G.D.Naidu.Published by Dr.G.D.Naidu Industrial labour welfare association ltd, Coimbatore.
6. Dr.R.Thyagarajan,(Gunapadam Thadhu Jeeva Vaguppu, Indian medicine and Homoeopathy, Chennai-106,Ed - 4 1992)
7. Sarakusuthiseimuraiyagal
8. International Journal of Advanced Multidisciplinary Research (IJAMR) ISSN: 2393-8870 www.ijarm.com Volume 3, Issue 6 -2016 34-38
9. International Journal of Advanced Research in Biological Sciences ISSN: 2348-8069 www.ijarbs.com Volume 3, Issue 6 – 2016 106-112
10. Brekhman I.I and Dordymov I.V New Substances of Plant Origin which Increase Nonspecific Resistance. Annual Review of Pharmacology 9 (1969).
11. Bhargava KP, Singh N. Anti-stress activity of *Ocimum sanctum*. Indian J Med Res 1981
12. Taur DJ, Nirmal SA, Patil RY, Karma MD. Antistress and antiallergic effects of *Ficus bengalensis* bark in asthma. Nat Prod Res 2007
13. Brekhman LI, Dardymov IV. New specific substance of plant origin, which increases nonspecific resistance. Ann Rev Pharmacol 1969; 9: 419-428.
14. Nadeem A, Chhabra SK, Masood A, Raj HG. Increased oxidative stress and altered levels of antioxidants in asthma. J Allergy Clin Immunol 2003; 111: 72-78.
15. MacNee W, Rahman I. Is oxidative stress central to the pathogenesis of chronic obstructive pulmonary disease? Trends Mol Med 2001; 7: 55-62.
16. Barry RH, Eugene DR, Theodore J, Antonuius VK. Total eosinophil count in the management of bronchial asthma. N Engl J Med 1975; 292(22): 1152-1153.

17. Oliver N, Gerald H, Gert K. Immunological and clinical changes in allergic asthmatics following treatment with omalizumab. Int Arch Allergy Immunol 2003; 131: 46-52.
18. Shimizu T, Shimizu S, Hattori R, Majima Y. A mechanism of antigeninduced goblet cell degranulation in the nasal epithelium of sensitized rats. J Allergy Clin Immunol 2003; 112: 119-125
19. Osama NO Joshi KR. Immunology: Immunology of Respiratory Disease. Agro Botanical Publishers, Bikaner, 1984; 321-9.
20. Lee-man C, But PPH, Yue D. Antiallergic and anti-inflammatory properties of the ethanolic extract of *Gleditisia sinensis*. Journal Pharmacy Pharmacology. 2002; 25: 9: 1179-82.

Table -1.Effect of ENC on Milk-induced leukocytosis

Groups	Diff.TLC(per cu mm)
I (Control)	4704.4± 144.5
II (Standard)	2019.4± 144.5**
III ENC LD(270 mg/kgbw)	4334.8±173.6
IV ENC MD(530 mg/kgbw)	2376.8± 199.2**
V ENC HD(800 mg/kgbw)	2103.6±210.1**

Table -2 Efffect of ENC on Milk-induced eosinophilia in mice

Groups	Diff.of Eosinophils (per cu mm)
I (control)	118.4± 2.34
II (standard)	63.6± 3.50**
III ENC LD	104.2±2.22**
IV ENC MD	80.6± 2.73**
V ENC HD	68.6±3.65**

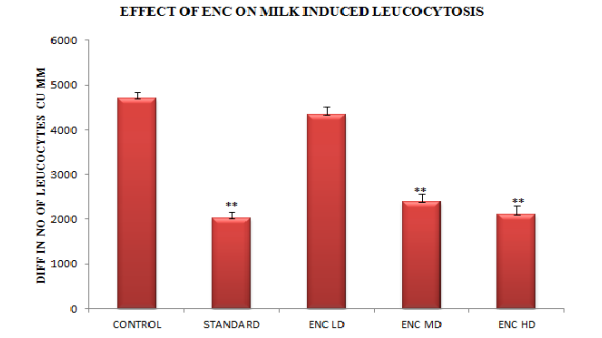

Fig.1 Effect of ENC on Milk-induced leukocytosis

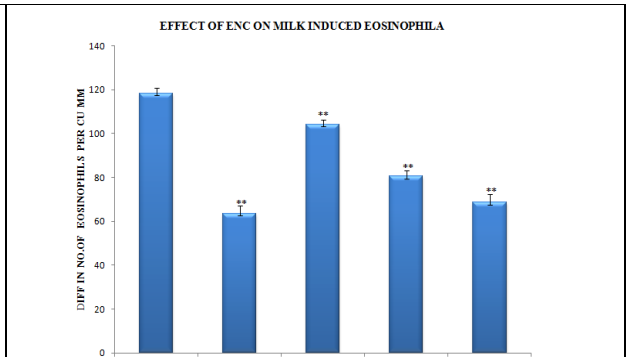

Fig.2 Effect of ENC on Milk-induced eosinophilia

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RESEARCH ARTICLE

Covid 19 Transformations in Indian Life Insurance Sector

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ABSTRACT

The Indian life insurance industry is the fifth biggest in the world. Although the per capita premium remains lower than in other emerging markets, India's working class remains one of the world highest, providing life insurance companies with a huge opportunity to expand into and throughout the Indian market. Due to the novel coronavirus outbreak in 2020, the life insurance firms are now providing cover against other infectious diseases. Despite the fact that the customer's viewpoint has shifted, insurance firms are being forced to reconsider their product strategies in the face of the pandemic. To maintain financial security, insurance firms around the world sell a variety of life insurance plans, including COVID-19-specific policies. In this paper we discussed about the performance of life insurance companies during COVID 19 and the growth of life insurance sector during covid-19.

Keywords: COVID 19, Life insurance, Pandemic, Premium, Market share, Policies

INTRODUCTION

Life Insurance Sector is one of the important sector in an economy. Which protect the interest of the individual as well as the business community at large and the society as a whole. Life insurance sector shows a noticeable transformation after the COVID 19. The COVID 19 Pandemic forced all the sector in an economy to modify their functioning and Indian life insurance sector also experienced the global vibration. The outcome of Covid Pandemic compelled the insurance providers to take rapid steps to withstand this shock. The report published by PWC clearly specified that the pandemic discouraged the insurance companies from spending substantially i.e, there are some places where they could consider investing. The disruption caused by the corona virus spread and the pandemic-induced lockdown resulted in the life insurance industry losing around million policies and premiums of around Rs

45, 000 Crore . Overall, the insurance sector lost four million policies and almost Rs 15,000 Crore in new company premiums. Since the lockdown happened, people were saving money for necessities. The higher official of LIC expressed the shortfall in the renewal premium during the pandemic period. The report further highlights that about Rs 30,000 Crore of renewal premiums did not materialize for LIC during the pandemic period. With the latest corona virus epidemic, more people are becoming aware of the importance of insurance. Many of them still see protection as a must to be prepared in the event of any potential unexpected events. This wasn't always the case, however. Prior to the COVID-19 epidemic in India, only 10% of people expressed interest in buying insurance to cover medical crises but now the scenario has changed and about 71% of the people felt the need for insurance.

Objectives of the Study

The major objectives of the study are

1. To provide an overview about life insurance business during COVID – 19.
2. To enumerate the recent changes in life insurance industry regarding the policies (plan).

RESEARCH METHODOLOGY

The present study is based on secondary data. Data and information have been extracted from IRDAI Annual report, IBEF and Bloomberg Quint's Calculation. The information collected has been classified, tabulated and analyzed as per the objectives for the study.

Review of Literature

Amlan Ghosh and Abhijit Mukherjee (2020) discussed about the impact on property-liability in India. The study through light to the policyholder and the insurers to take a holistic view of the impact of COVID - 19 situation and plan for the future accordingly. Susan Holliday and Prapti Sherchan (2020) stated the insurance industries best practices on how to engage and support women clients and agents. They also explain about women contribution towards the success of the insurance industry and how best to engage with them during the crisis. Ravi Shekhar (2020) elucidated the COVID - 19 impact on all major stakeholders including hospitals, Insurers, Corporate and continuously changing claim patterns in pre and post COVID time. They also discussed about the need of the hour to explore forward looking measures to mitigate the uncertainty and be ready for future impacts with digital changes. Babuna P, Yang, X., et al (2020), investigated the impact of COVID-19 on the insurance industry by studying the case of Ghana from March to June 2020. They used qualitative and quantitative interview to estimate the impact of the pandemic. In their study they compared and forecasted the normalization of economic indicators from January 2021. They concluded that insurance companies were affected by different factors such as liquidity, portfolio at risk, reliance on reinsurance, level of free assets and protection that reinsurers have in place. Kannamani Ramasamy (2020) discussed about the factors such as lockdown approach, moratorium, and different impacts in banking, financial services and insurance sector. They also stated that every industry is struggling to manage and plan for continuous improvement through various initiatives.

Life Insurance Industry in COVID 19

There was a feeling of panic and apprehension among the population within a few weeks of the start of the COVID-19 pandemic, as the country reported over a dozen deaths due to coronavirus infection. On the recommendation of the IRDAI, insurers have explained that death caused by COVID-19 will be considered as a general death, and the allegation will be admissible if the coronavirus was discovered after the policy was issued. During the time of crisis, this measure guaranteed policyholders' peace of mind. Due to the outbreak of pandemic situate people have certainly become more aware of the value of insurance and especially term and health insurance policies. The term life insurance industry began to gain momentum within a month of the COVID-19 pandemic. In order to ensure that consumers would purchase insurance from the comfort of their own homes, insurers began offering policies via telemedicine instead of a physical examination. This meant that even though people followed the lock-down rules

and remained at home, they were still protected by the measures taken by the life insurers. Table 1 and 2 represents by the life insurance premium received by the life companies during 2016-2021.

The above table depicts the life insurance premium received by the life insurers during 2016-2021. It is interesting to note from the table that year –on-year basis the new business life insurance premium received by the life insurance companies in India shows a fluctuating trend. The new business premium received in 2016 stands at US$ 21.5 billion and it declined to US$ 1.0 billion in 2021.Though people are very keen to take life insurance policy due to lockdown many people suffered a lot to lead their livelihood. So it is not possible to take new insurance policies. It is noticed from the data that even the renewal premium also reduced to a greater extent from US$ 35.3 billion to US$ 30.6 billion in 2020. The present scenario of life insurance business is not appreciable.

Decline on Life Insurer's New Business Premium

The damage caused by the outbreak of COVID-19 and the resulting lockdown imposed by the government to curb its spread resulted in a 32 percent drop in new business rates for life insurance firms in March. Life insurance firms' new business premiums in March totaled Rs.25,409 Crore, compared to Rs 37,459 Crore in March 2019. In March 2020, the new business premiums of 23 private life insurers fell by 34.21 percent to Rs 8,342 Crore compared to Rs.12,682 Crore in the same period the previous financial year. In the same month, the state-owned life insurance behemoth Life Insurance Corporation (LIC) saw its premiums drop by 31.11 percent. It raised by Rs 17,066 Crore in new business premiums in March 2020, compared to Rs 24,776 Crore in March 2019. Life insurance, on the other hand, saw new company premiums rise 20.6 percent to Rs 2.58 trillion in FY20, relative to Rs 2.14 trillion in FY19. In FY20, LIC outperformed private insurers in terms of new business premium development. The LIC premium increased by 25.17 percent to Rs 1.77 trillion, while private insurers saw an increase of 11.64 percent to Rs 80,919 Crore.

Premium Market shares of life insurance companies

Figure 2 represents premium market shares in first year life insurance. During the FY 2020, there were 24 private players in the life insurance market, compared to just four in FY 2002. Life Insurance Corporation of India, only public sector life insurer, retained its market leadership with approximately 53% of the new business market share in FY 2020. In FY 2020, HDFC Regular Life Insurance led the private sector lenders in new business premiums with a market share of over 14%, followed by SBI Life Insurance (9%), and ICICI Prudential Life Insurance (6%).

Changes in Term Insurance Trends due to COVID 19

The coronavirus pandemic has pressured many businesses in a variety of industries to change their business practices. The insurance industry is not an exception to it.

Cover for Pandemics: 2020 has been a difficult year for many, with the coronavirus ravaging the world causing a pandemic. Many insurers have begun to sell insurance from viruses-related diseases in order to ensure that policyholders remain covered in the future. This can be seen as a term insurance adjustment.

Customer forced Solutions: Previously, insurance firms took a more one-size-fits-all strategy, but this is no longer the case. Customers are more knowledgeable than ever before, and they want options that are tailored to their specific needs. Insurers are also selling custom-made plans in order to satisfy consumer needs. This is one of the term insurance patterns that will continue to emerge in the future.

Digital Access: With the number of coronavirus cases rising every day, there has been a significant increase in online purchases and deals. People are increasingly purchasing goods and services online, including insurance. In the term insurance market, this is a significant change. Policyholders with online insurance may make payments and upload documents from the protection and convenience of their own homes.

Enhanced Claim Setting: Insurance firms will see an improved claim resolution process in 2020, in addition to digitalization and customer-centric solutions. Claims can now be filed more quickly, and policyholders can easily upload the relevant documents. Insurers can do this to boost their productivity and resolve the claims more quickly and efficiently.

Increased Options and Benefits: When the number of potential policyholders rises, so will the level of competition among insurers. As a result, various types of regulations, as well as riders and special benefits, are implemented. Insurance firms can try to outperform the market by providing potential consumers with additional benefits.

Growth of Insurance Sector

The reopening of the economy helped private life insurance firms raise premiums at pre-pandemic rate. According to Bloomberg Quint's estimate based on disclosures, total new business premium—individual and community policies—returned to pre-covid levels in October, with Rs 22,776 crore worth of policies underwritten. This was the highest level in more than nine months. Private life insurers' annualized premium equivalents grew by 14% year on year, basis the highest in nine months, while the Life Insurance Corporation of India, the country's largest life insurer, saw a 6% rise. The industry as a whole saw an 11% increase in first-year premiums. As business returned to normal, the higher sales through bancassurance or banking partners, which is the largest distribution channel for life insurers, aided the results. Bank branches were not completely open after the lockdown, walk-ins were down, and insurers pushed for digital delivery. After the Covid-19 pandemic, the selling of life insurance policies has grown steadily on the internet. Multiple insurers reported in their post-earnings statements that business is picking up because of the pandemic's increased awareness of insurance. Although demand for all product categories has increased, the security market, which includes term plans, is leading the way.

CONCLUSION

The Covid-19 pandemic in 2020 has encouraged numerous insurance sectors to create novel ideas and deals that have never been considered before. As a result, advanced and updated trends have evolved to meet the needs of consumers. As a result, numerous forms of policies with added benefits have arisen. Insurance is a form of protection. The insurance industry has made strategic attempts to navigate the unforeseeable business environment by creating new products, which is a major investment and advantageous during a climacteric era like the Covid-19. Other factors, such as the increasing middle class and young population, as well as security from an uncertain future and retirement planning, have aided the cause.

REFERENCES

1. Amla Ghosh and Abhiit Mukherjee, COVID-19 and property- Liability Insurance in India: Impact and prospect, Munich Personal RePEc Archive, 2020,
2. Babuna, P., Yang, X., Gyilbag, A., Awudi, D. A., Ngmenbelle, D., & Bian, D. (2020). The impact of Covid-19 on the insurance industry. *International journal of environmental research and public health*, *17*(16), 5766.
3. Ramasamy, D. (2020). Impact Analysis in Banking, Insurance and Financial services industry due to COVID-19 Pandemic. *Pramana Research Journal*, *10*(8).
4. Ravi shekhar and Sivangi Pandey, Adjusting to the new Normal impact of COVID-19 in Insurance Industry, 2020, pp: 1-9.
5. Susan Holiday, Prapti Sherchan and Sarah Ebrahimi, COVID-19 and the insurance industry: Why a Gender-Sensitive Response Matters. International Finance Corporation (2020).
6. COVID 19 Impact Retrieved from https://www.financialexpress.com/money/insurance/how-can-insurance-companies-stay-relevant-in-times-of-covid-19/2002725.
7. Changing term Insurance Plan Retrieved from https://www.aegonlife.com/insurance-investment-knowledge/covid-19-impact-on-insurance.
8. COVID 19 lose in premium Retrieved from https://www.business-standard.com/article/pf/covid-19-impact-life-insurers-lose-4-mn-policies-rs-45-000-cr-in-premiums-120091601735_1.html
9. Decline of Life insurance new business premium Retrieved from https://www.business-standard.com/article/companies/covid-19-impact-life-insurer-s-new-business-premium-declines-32-in-march-120042400011_1.html

10. Premium Market share Retrieved from https://www.ibef.org/download/Insurance-June-2020.pdf
11. https://www.capgemini.com/2020/10/life-insurance-distribution-optimization/
12. Performance during COVID 19 Retrieved from https://www.bloombergquint.com/business/private-life-insurers-grow-at-fastest-pace-in-nine-months-return-to-pre-covid-levels.
13. Growth of Insurance Sector Retrieved from http://www.businessworld.in/article/New-Trends-Emerge-in-Life-Insurance-Industry/20-08-2020-310971/

Table 1: Life Insurance Premiums Received by the Life Insurance Companies during 2016-2021

(US$ billions)

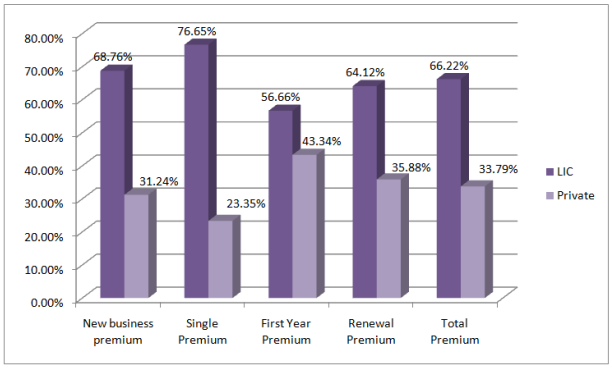
Years	New Business premium	Renewal Premium
2016	21.5	35.3
2017	27.2	37.7
2018	30.1	41.0
2019	30.7	42.0
2020	37.0	30.6
2021	1.0	-

Source: IRDAI annual report (2019-2020)

Table 2: Performance during COVID 19 Year 2020

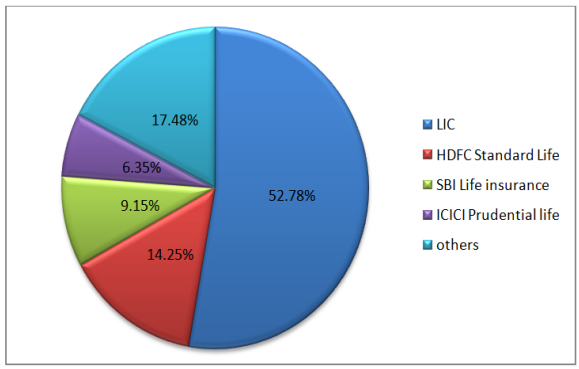
Month	LIC	Private	Other Industry
January	99%	10%	46%
February	-7%	4%	-1%
March	-64%	-40%	-50%
April	-48%	-40%	-44%
May	-3%	-32%	-20%
June	8%	-7%	-1%
July	10%	-7%	-0.3%
August	2%	-6%	-2.3%
September	5%	4%	5%
October	6%	14%	11%

Source: Bloomberg Quint's Calculation



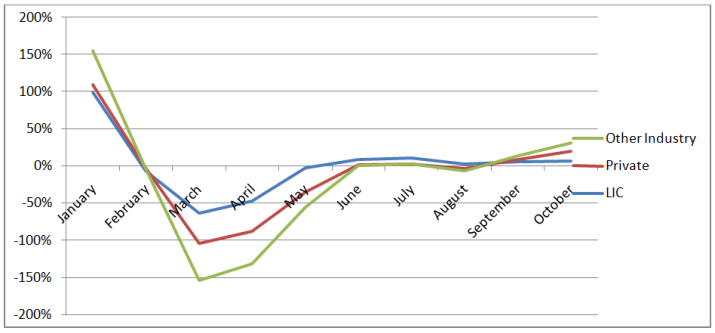
Source: IRDAI Annual report 2019-2020

Figure 1: Life Insurance Premiums received by the Life Insurance Companies during 2019- 2020



Source: IBEF

Figure 2: Premium Market Shares in First Year Life Insurance FY 2020



Source: Bloomberg Quint's Calculation

Figure 3: Performance during COVID 19 Year 2020

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RESEARCH ARTICLE

Luteolin as a Potential Inhibitor of Nadph Oxidase - An *In silico* Analysis

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ABSTRACT

NADPH oxidase (NOXs) is the enzyme that plays an important role in the generation of reactive-oxygen-species and thereby found to be upregulated in various oxidative stress disease conditions. Flavonoids are secondary metabolites having anti-oxidative properties. Among them, Luteolin, 3,4,5,7- tetrahydroxyflavone, which is found in many fruits and vegetables and in some of the medicinal plants possesses antioxidant properties. So the objective of this *in silico* study is to identify whether luteolin be effective in influencing the NADPH Oxidase enzyme activity by binding to its subunits using AutoDock 4.2. The chemical constituents were retrieved from PDB and PubChem database. The molecular and bioactive properties of ligand have been predicted using Molinspiration online server. It indicated that luteolin satisfies Lipinski's rule of five and can be used as potent inhibitor or drug. The binding energy value and the hydrophobic interactions obtained in the current study confirmed that there is a strong binding affinity between luteolin and NADPH oxidase enzyme that might be used to inhibit the activity of the enzyme in oxidative stress conditions. Therefore, luteolin may be used as potent drug and further may reduce the complications of oxidative stress diseases.

Keywords: Luteolin, NADPH Oxidase, Oxidative stress diseases,Molecular Docking, Molinspiration, Lipinski rule.

INTRODUCTION

Oxidative stress is a molecular deregulation in reactive oxygen species (ROS) metabolism which is involved in the pathogenesis of several diseases. The ROS are produced by certain enzymes, like nitric oxide synthases, NADPH oxidases, cytochrome P450 reductase, and xanthine oxidase. Among these enzymes, NADPH oxidase is the only enzyme whose specific function is to produce reactive oxygen species [1]. NADPH oxidase enzyme coordinates in cell damage, stress response and regeneration of tissues. Increased level of reactive oxygen species produced due to hyperactivity of NADPH can lead to genetic instability followed by excessive proliferation, DNA damage activation and proliferative senescence leading to apoptosis [2],[3],[4]. With its prototypical role in ROS biology and redox signalling, NADPH oxidase can be used as a attractive drug target in oxidative stress disease.

Several studies have reported that flavonoids have anti-oxidatant, anti- inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with its capacity to modulate key cellular enzyme functions. It alsoacts as potent inhibitor for enzymes like, xanthine oxidase, cyclo-oxygenase, lipooxigenase and phosphoinositide3-kinase [5].Among the flavonoids luteolin (30,40,50,70-tetrahydroxyflavone, Lut) is one of the most prevalent flavonoidwhich act as a antioxidant, free radical scavenger. Hence, it has been hypothesised that luteolin may bind to NADPH oxidase and thereby inhibit its activity. The objectives of the study were to perform docking between luteolinand NADPH Oxidase by using AutoDock 4.2, to find out the drug like property of luteolin and also to study the molecular interaction between luteolin and NADPH oxidase .

MATERIALS AND METHODS

Target protein identification and preparation

The NMR structure of NADPH oxidase $P22_{Phox}$-$P47_{Phox}$ Complex was downloaded from Protein Data Bank (PDB) database and the PDB ID is 1WLP. The protein was preprocessed using AutoDock 4.2 to remove all water molecules and add hydrogen atoms and electronic charges were assigned to the protein atoms using kollman united atoms.

Ligand Preparation

The 3-dimentional structure of Luteolin (PubChem CID: 5280445) was retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in 'SDF' format, followed by preparation 'PDB' format using OpenBabel tool. The Canonical SMILES (C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O) format was saved.

Molecular Descriptor Calculation

Molinspiration online database (https:// www.molinspiration.com) was used to calculate the molecular descriptors of luteolin, such as logP, polar surface area (PSA), molecular weight, number of atoms, number of O or N, number of OH or NH, number of rotatable bonds, volume, drug likeness includes G protein couples receptors (GPCR) ligand, ion channel modulator, kinase inhibitor and nuclear receptor ligand, and number of violations to Lipinski's rule of Five.

Lipinski's rule of Five

Lipinski's rule of five is a rule to evaluate the drug likeness, or to find out a chemical compound with a certain pharmacological or biological activity has properties that would make it as a orally active drug for consumption in humans[6]. The rule states that:

i) Molecular weight <=500g/mol
ii) Number of hydrogen bond acceptors <=10
iii) Number of hydrogen bond donors <=5
iv) Number of rotatable bond <=10
v) Partition coefficient LogP<=5

Molecules violating any of these rules may have problems with bioavailability.

Molecular Docking

The Blind molecular docking of target protein and ligand was performed using Auto Dock 4.2 (https://autodock.scripps.edu/resource/tools) software. To the protein molecule hydrogen atoms and kollman charges were added. The modified protein was saved in PDBQT format for further Autodock calculation. The grid box of size 202 x 162 x 138 □ covering the whole protein was constructed and docking was carried using Lamarckian genetic algorithm. After docking the 'dlg' file was used to identify the best pose of ligand based on the binding energy. The pose with lowest binding energy was selected to visualize the ligand-protein interaction. The molecular

interactions were visualized using Biovia Discovery Studio Visualizer. Default ligand-receptor tool was used to determine the hydrogen and hydrophobic interactions.

Interaction Profiling

An interaction profile is a set of values and keys that represents the interaction features occurring in a protein-ligand complex. The keys represent each feature and the value is a quantitative measure of the feature. These features are based on the structure of the ligand, the residue properties of the interacting residues and the non-covalent intermolecular interactions. It was calculated using Protein-Ligand Interaction Profiler (PLIP) python package.

RESULTS

Molecular Descriptor Calculation and Lipinski rule of five

The present study was carried out to understand the drug-likeness character of luteolin and its binding affinity with NADPH oxidase enzyme. The molecular descriptor values and the drug likeness properties of luteolin were shown in table 2.

Molecular Docking analysis

Molecular docking is a one of the important part in the process of drug design, which is carried out in this study to evaluate the binding efficiency of the luteolin against target NADPH oxidase (1WLP) using AutoDock 4.2 software. The visualized hydrogen interactions between luteolin and NADPH oxidase using Biovia Discovery Studio Visualizer were shown in figure 1. This study revealed the molecular interaction of Lut with Lys169, Glu168, Asp166, Pro16, Ala18, Pro17, Thr170, Tyr167, Pro14, Arg15 and Phe209 amino acids of protein 1WLP. The binding energy of first three rank was shown in table 5. The least negative value of the binding energy (-5.75 kcal/mol) indicates the strong interactions of Lut with 1WLP protein.

Protein-Ligand Interaction Profiler (PLIP)

PLIP was used to describe the protein-ligand complex according to their molecular interactions. After docking 1WLP with luteolin the details of molecular docking was analysed using PLIP online server and the results were shown in table 4.

DISCUSSION

In the present study the target protein NADPH oxidase $P22_{phox}$-$P47_{phox}$ complex and the ligand luteolin was retrieved from the database. Using molinspiration online software the molecular property and bioactivity activity of luteolin was calculated. Molecular docking was performed to find the binding efficiency for luteolin against NADPH oxidase. The advantage of choosing NADPH oxidase is because it is the only enzyme that is involved in the production ROS and become an attractive drug target for the study. Therefore, inhibition of the NOX enzyme in disease can aid to alleviate the diseased condition. It was found that $p47_{phox}$ is a regulatory subunit of NADPH oxidase which produces superoxide. This$p47_{phox}$ phosphorylation and assembly with $p22_{phox}$ activates the enzyme. Many studies have reported the most of the phytochemicals from herbal sources especially flavonoids have the antioxidant property which can act as a good agent in drug designing. The effect of a drug in human beings can be understood by its pharmacological activities. The main aim of a drug is to bind to its biological target. It was found thatluteolin satisfying lipinski's rule of 5 without violating any rule making it as a potent drug. The Topological polar surface area (TPSA) is a useful parameter for the prediction of transport of drug. TPSA value of luteolin was 111.12 □ which is below 140 □ thus indicating it as a better drug.

The bioactivity score of a complex was calculated using different parameters like G-protein-coupled receptor ligand, nuclear receptor ligand, kinase inhibition, ion channel modulation, protease inhibition and enzyme activity

inhibition. To become a biological active compound the bioactivity score should be as follows: if it is more than 0.0 then it is active, if it is between -5.0 to 0.0 then it is moderately active and if it is less than -5.0 then it is inactive. The bioactivity score of luteolin was between -5.0 to 0.0, indicating it as active drug [7]. In the study AutoDock4.2 tool was used for docking to explore and validate the key interactions between Lut with NADPH oxidase. The lowest binding energy of about (-5.75 kcal/mol) showed the greater affinity of the Lut against NADPH oxidase further using of it as potent inhibitor. The molecular interaction of Lut with Lys169, Glu168, Asp166, Pro16, Ala18, Pro17, Thr170, Tyr167, Pro14, Arg15 and Phe209amino acids of 1WLP was predicted using Biovia Discovery Studio Visualizer. Further, Protein-Ligand Interaction Profile was carried out and the distance between the ligand and interacting amino acid with its chain were found.

A study was conducted by Rajapriya *et al* on molecular interaction of Lut as a potent anti-inflammatory agent with procaspase-1, ASC-CARD and ASC-PYD.The results of *in vivo* studies showed that Lut play major role in reducing inflammation by manipulating ASC levels and thereby inhibiting caspase-1 activation in pancreatic acinar cells which inturn stops the activation of pro-inflammatory cytokines. The in-silico analysis showed that luteolin binds efficiently with target protein Procaspase-1, ASC-CARD, and ASC-PYD reducing the formation of pro-inflammatory cytokines and prevent pancreas tissue injury [8]. Yan *et al* studied inhibitory effect of luteolin on Xanthine oxidase (XO) and its interaction mechanism was evaluated by multispectroscopic method coupled with molecular stimulation. The results showed that luteolinreversibily inhibits XO and it binds with a single binding site driven mainly by hydrophobic interaction [9].

In a study conducted by Jiang *et al*, the interaction between apocynin analogues and NADPH oxidase was analysed using docking studies. The apocynin analogues were docked with NADPH oxidase (1K4U) and the results showed that the Pi interaction between this two has direct contribution to the inhibition of the enzyme [10]. Lu *et al* examined the effect of luteolin on diabetes-induced oxidative stress and inflammation in the retina of rats. They have suggested that luteolin can be effective for protection against diabetes-induced retinal neurodegeneration by inhibiting the levels of inflammatory markers and oxidative stress [11].

In conclusion, luteolin was found to be a best drug candidate against NADPH oxidase. It can inhibit the NADPH oxidase activity in oxidative stress diseases efficiently. In this *in silico* study, luteolin passed all the essential tests required for a potent drug. Based upon the pharmacokinetic analysis and docking study,luteolin was effective in the inhibition of the enzyme. Therefore, luteolin may be used as potent drug and further may reduce the complications of oxidative stress diseases.

REFERENCES

1. K. Block, Y. Gorin, “Aiding and abetting roles of NOX oxidases in cellular transformation”, National Review Cancer. 12. 627–637, 2012.
2. U. Weyemi, O. Lagente-Chevallier, M. Boufraqech, M.Prenois, F. Courtin, B. Caillou, “ROS-generating NADPH oxidase NOX4 is a critical mediator in oncogenic HRas- induced DNA damage and subsequent senescence”, Oncogene 31,1117–1129, 2012.
3. M. Ogrunc, R. Di Micco, M.Liontos, L.Bombardelli, M. Mione, M.Fumagalli, “Oncogene-induced reactive oxygen species fuel hyperproliferation and DNA damage response activation”, Cell Death Differ. 21, 998–1012, 2014.
4. H.Q. Ju, H. Ying, T. Tian, J. Ling, J. Fu, Y. Lu, et al, “Mutant Kras- and p16- regulated NOX4 activation overcomes metabolic checkpoints in development of pancreatic ductal adenocarcinoma”, Nat. Commun. 8, 14437, 2017.
5. E. Walker, M. Pacold, O. Perisic, “Structural determinations of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine”, Mol Cell 6, 909–919, 2000.
6. C.A. Lipinski, “Lead- and drug-like compounds: the rule-of-five revolution”. Journal of Drug Discovery Today: Technologies, 1(4):337-41, 2004.

7. A.M. Dar, M.A. Khan, S. Mir, M.A. Gatoo, "DNA binding, cleavage activity, molecular docking, cytotoxicity and genotoxicity studies of newly synthesized copper based metal complexes". Pharm Anal Acta 7:464, 2016.
8. S. Rajapriya, A. Geetha, K.G. Kripa, "Modulating effect of luteolin on ethanol and cerulean induced inflammation- *In silico* and *in vivo* studies using rat model of pancreatitis", Indian Journal of Experimental Biology, Vol.57, pp 486-496, 2019.
9. J. Yan, G. Zhang, Y. Hu, Y. Ma, "Effect of luteolin on xanthine oxidase: Inhibitionkinetics and interaction mechanism merging with docking simulation", Food Chemistry, 2013.
10. J. Jiang, H. Kang, X. Song, S. Huang, S. Li and J. Xu, "A Model of Interaction between Nicotinamide AdenineDinucleotide Phosphate (NADPH) Oxidase and ApocyninAnalogues by Docking Method",International. Journal of Molecular Science, 14, 807-817, 2013.
11. H. Lu, Y. Chen, X.B. Sun, B. Tong, &X.H. Fan, 'Effects of luteolin on retinal oxidative stress and inflammation in diabetes', RSC Advances, 5(7), 4898–4904, 2015.

Table 1: Ligand Parameters

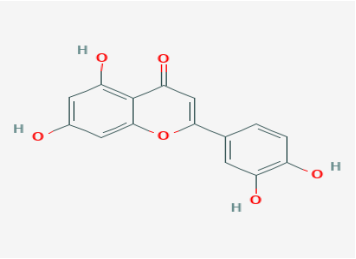
Compound	IUPAC Name	Pubchem CID	Molecular Formula	2D Structure
Luteolin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one	5280445	$C_{15}H_{10}O_6$	

Table 2: Molecular Descriptor and Drug likeness property of Luteolin

Properties	**Luteolin**
Polar surface area (TPSA)	111.12
Molecular volume	232.07
GPCR Ligand	-0.02
Ion channel modulator	-0.07
Kinase inhibitor	0.26
Nuclear receptor ligand	0.39
Protease inhibitor	-0.22
Enzyme inhibitor	0.28

Table 3: Lipinski Rule of five

Compound	No. of hydrogen bond donors	No. of hydrogen bond acceptors	Molecular weight (g/mol)	LogP	Number of rotatable bonds
Acceptable Values	<5	<10	<500	<5	<10
Luteolin	4	6	286.24	1.97	1

Table 4: Protein-Ligand Interaction Profiler

Hydrophobic Interactions					
Index	**Residues**	**Amino acid**	**Distance**	**Ligand Atom**	**Protein Atom**
1	14A	PRO	3.84	1540	113
2	14A	PRO	3.35	1532	114
3	15A	ARG	3.93	1539	120
4	167B	TYR	3.73	1534	397
5	209B	PHE	3.04	1536	802

Hydrogen Bonds									
Index	**Residue**	**AA**	**Distance H-A**	**Distance D-A**	**Donor Angle**	**Protein donor?**	**Side chain**	**Donor Atom**	**Acceptor atom**
1	16A	PRO	2.53	3.26	131.73	x	x	1543(O3)	136(O2)
2	16A	PRO	2.07	2.67	117.67	x	x	1541(O3)	136(O2)
3	18A	ALA	2.87	3.53	125.16	✓	x	147(Nam)	1541(O3)
4	166B	ASP	2.19	3.08	151.10	x	x	1545(O3)	385(O2)
5	168B	GLU	1.78	2.71	158.38	✓	x	405(Nam)	1545(O3)
6	169B	LYS	2.22	3.15	160.11	x	x	1547(O3)	418(O2)

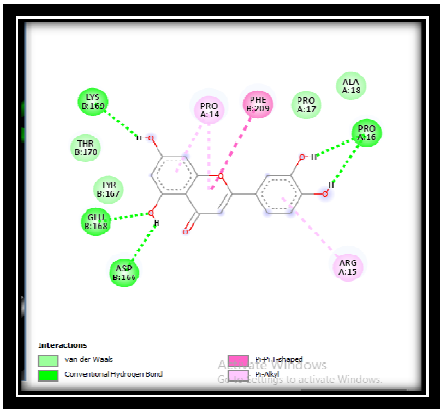


Figure 1. 2D interaction between the protein-ligand complex. Figure showing the Lut contact with NADPH oxidase (1WLP) after molecular docking. Different bonds are shown in different colours.

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RESEARCH ARTICLE

Antimicrobial Activity of Siddha Formulations against Enteric Pathogens

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ABSTRACT

An enteric pathogen may be identified as any microbe that is able to cause enteric disease whose pathogenesis can involve direct invasion, host inflammation, and secrete toxins that damage the host cells. Enterobacteriaceae family includes coliforms like *E. coli, Salmonella, H. pylori, Shigella flexneri* and other gram negative pathogens that can be a component of normal enteric flora as a commensal or as enteric pathogens. As a result of the emergence of these multidrug resistant microbes, new treatment methodologies are being searched continually**.** Siddha, one of the ancient Indian traditional systems of medicine has come into limelight for its therapeutics prepared from natural resources. The Siddha system of medicine prescribes various herbomineral formulations to alleviate gastro intestinal disorders. The present experimental study is to evaluate the antimicrobial properties of ten herbo-mineral siddha drugs on isolated microbial strains of enteric pathogens. Seven isolated microbial pathogens *E.coli*, *Proteus mirabilis*, *H. pylori*, *Shigella flexneri*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perfringens* were cultured and inoculated into sterile nutrient broth. The susceptibility of the microorganisms to ten Siddha drugs Elathy Chooranam (G1), Gunmakudori Mezhugu (G2), Athimathura Chooranam (G3), Sangu Parpam (G4), Kukkil Parpam (G5), Silasathu Parpam (G6), Venpoosani Legium (G7), Thaleesathi Chooranam (G8), Thriphala Chooranam (G9) and Thriphala Karpam (G10) was screened by Agar well diffusion method and the Minimum inhibitory concentration (MIC) of the effective drug was calculated by Broth dilution method. Among the ten Siddha medicines screened, G1 was considered as a potential Siddha formulation against all the ten enteric pathogens. The Drug Elathy Chooranam (G1) is validated scientifically to be an efficient antimicrobial Siddha formulation against enteric bacterial infections caused by the above tested pathogens.

Keywords: Siddha, Enteric pathogens, Antimicrobial activity, Agar well diffusion method, Minimum Inhibitory Concentration, broth dilution method

INTRODUCTION

Enterobacteriaceae include coliforms like *E. coli, Salmonella, H. pylori, Shigella flexneri* and other gram negative pathogens that can be a component of normal enteric flora as a commensal or as enteric pathogens. An enteric pathogen may be classified as any microbe that is able to cause enteric disease whose pathogenesis can involve direct invasion, signals triggering, host inflammation, secrete toxins that damage the host cells and exploitation of other environmental factor associated to thrive in the host [1]. Enteric infections can result in profound effects on intestinal absorption, nutrition and childhood growth and development and affects the global mortality rate. It is estimated that globally, infectious diseases are the most important cause of death accounting for approximately one-half of all deaths in tropical countries of which typhoid, an enterobacterial infection causes 200,000-600,000 deaths each year [2].

Emergence of Drug Resistance Strains

Antibiotics are bactericidal or bacteriostatic chemotherapeutic agents which are required for the management of microbial infections. The optimism of antibiotic discovery claiming them as “magic bullets” to tackle these microbes has been tampered by the emergence of bacterial strains that are resistant against existing antibiotics. Drug resistance refers to a decrease in the efficacy of a drug for a disease as a result of which it is increasingly being difficult to treat even common illnesses. As a result of the emergence of these multidrug resistant microbes, new treatment methodologies are being searched continually to treat them in a better way [3].

Pathobionts

Antibiotic resistance in enteric pathogens is a major cause of concern for certain life threatening enteric diseases. Antibiotic Resistant strains that emerge from commensal organism are known as Pathobionts. There is a continual appearance of Antimicrobial Resistance and Multidrug Resistant Organisms in enteric pathogens like Campylobacter, Shigella, Salmonella, Vibrio cholera, E. coli and Enterococci **[4].** Emergence of these antimicrobial resistance strains is a common event in a mixed ecosystem like gut. Various mechanisms attribute to the emergence of Antimicrobial Resistant strains out of which enzymatic modifications to the antimicrobial agent is the key one. The genes that are responsible for this resistance occur in mobile genetic elements, which get transferred to the cells through horizontal gene transfer from cell to cell in such a complex ecosystem**. [5].** In a study conducted in India on the multidrug resistant enteric pathogens, it was found that the traits for resistance is found on the mobile genetic elements **[6].** Hence in the present day circumstances, natural herbs and medicines are increasingly gaining acknowledgement in treating diseases.

Siddha System of Medicine

Siddha, one of the ancient Indian traditional systems of medicine has come into limelight for its therapeutics prepared from natural resources. Siddha has several categories of drugs derived from herbs, minerals, animal and marine products. Many of their constituents possess relevant pharmacological properties that can be used as an alternative tool against these resistant strains of microbes. or can also be used synergistically as they act as immune modulators, have potential antimicrobial action, modify the antibiotic resistance and also provide symptomatic relief [7].

Antimicrobial Activity

The antibacterial and antifungal activity of many Indian medicinal plants has been carried out by broth dilution assay and their Minimum Inhibitory Concentrations were determined [8]. As there are only a limited number of researches in the herbomineral formulations of Siddha, it is the need of the hour to evaluate the scientific basis for the

traditional use of these formulations and to establish the antibacterial potential of many Siddha medicines. The present study involves the evaluation of antibacterial potential of ten Siddha formulations namely G1, G2, G3, G4, G5, G6, G7, G8, G9 and G10 against seven major enteric pathogens *E.coli*, *Proteus mirabilis*, *H. pylori*, *Shigella flexneri, Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perfringens.*

MATERIALS

Siddha Medicines

Authenticated drug samples of G1, G2, G3, G4, G5, G6, G7, G8, G9 and G10 were procured from local market, Chennai. All these commercially available Siddha formulations are in current clinical practice in order to treat the gastrointestinal infections. The ingredients, dosage forms and indications of these medicines **(Table 1.)** are mentioned in Siddha literature Gunapadam (Siddha Materia Medica).

Equipments

The equipments required for the study were Autoclave, Incubator, Zone reader, DMSO, Well maker, Micropipette, Conical flasks, Petri dishes, Test tubes, Beakers and Sterile tips.

Medium

The media used for the study were Nutrient agar, Nutrient broth and Muller Hinton Agar (MHA). Nutrient agar and Nutrient broth were used for culturing the organisms and MHA was used for the antibacterial assay.

METHODS

Cleaning and sterilization

All the Glass equipments used in the present study were cleaned with cleaning solution and sterilized in hot air oven at 180^{0}C for 3 hours. All the media used were sterilized by autoclaving at 121^{0}C at 15psi for 15-20 minutes.

Preparation of extract

The extracts of the ten Siddha medicines were prepared at different concentrations by serial dilution technique. The stock solution of 1% of each drug was prepared by dissolving 0.1gram of the drug in 10ml of distilled water. All the samples were tested at 250µg/ml based on standard protocol.

Culture of Pathogens

The bacterial pathogens *E.coli*, *Proteus mirabilis, H. pylori*, *Shigella flexneri*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perferigens* were procured from MTCC, IMTech, Chandigarh, India and they were subcultured on Nutrient agar (Himedia laboratories, Mumbai) incubated at 37°C for 18 hours and stocked at 4°C. The pathogenic bacteria culture was inoculated into sterile nutrient broth and incubated at 25°C for 48-72 hours until the culture attained a turbidity of 0.5 McFarland Standard units.

Agar well diffusion Assay

Agar well diffusion method was used to test the extracts of the chosen ten Siddha medicines for their antimicrobial activity using Mueller Hinton Agar **[9].** The agar was poured onto a medium sized petriplate. 100µl of 500mg Tetracyclin and Fluconazole were pre inoculated on to the plates in order to prevent the growth of undesirable bacterial or fungal contaminants and the agar was allowed to solidify. Lawn culture of the chosen enteric pathogens was spread on to their respective agar plates. Wells of 7mm diameter were punched on the solidified agar using well maker. The wells were marked for different concentrations of the extracts and 50µl of the extracts of the prepared concentrations were filled into each well. Tetracycline was used as the positive control and the plates were then

incubated at 37°C for 24 hours. After the incubation period, the Zones of inhibition of each medicinal extract were measured.

Determination of Zone of inhibition and Minimum inhibitory Concentration (MIC)

The Zone of inhibition was obtained by measuring the clear zone around each extract, from the agar well to the end of the zone by zone reader and the values were noted in millimeter (mm). Strains with well marked inhibition zones were considered as the ones sensitive to the dug, and those without the clear inhibition zone were considered as resistant strains. Broth dilution method in 96 well microtitre well plates was used for determining the Minimum inhibitory Concentration (MIC) of the extracts. Serial dilution of the extracts was made and 0.5 McFarland Standard units of the organisms were inoculated into the diluted extracts. The 96 microtitre well plate was then incubated at 37°C for 24 hours and MIC value was read [10].

RESULTS

The present study shows that among the ten Siddha formulations that were screened for antimicrobial activity against the seven enteric pathogens, G1 (Elathy Chooranam) was established to have significant antimicrobial activity with considerable zone of inhibition against six of the tested pathogens (Fig. 1). Also G2 (Gunmakudori Mezhugu) and G9 (Thriphala Chooranam) do have antimicrobial potential as they evidenced for zone of inhibition against *Staphylococcus aureus*, *Shigella flexneri* (Fig. 2). Thriphala Chooranam was also found to have activity against *Clostridium perfringens* (Fig. 3). G4 (Sangu Parpam) showed more specific activity against *Streptococcus pyogenes*. The formulations G2, G3, G5, G6, G7, G8, and G10 though used for gastro intestinal disorders in Siddha system of medicine, they showed lesser antimicrobial activity against the tested enteric pathogens. MIC was determined using the broth dilution method for the tested medicines G1, G2, G4 and G9.

DISCUSSION

Many bacteria causes gastrointestinal diseases out of which enteric pathogens like *E.coli, Salmonella, Shigella, Clostridium, Vibrio and Staphylococcus* plays a major role in causing diseases like diarrhea, enteric fever, shigellosis and food poisoning and cholera. All these diseases occur as a major outbreak globally. These intestinal illnesses have become a major public health concern in many countries. WHO reports that diarrheal disease follows age associated pattern causing morbidity in children in their early stages of life (WHO 2008). Enteric fever shows age pattern of incidence wherein the highest hospitalization rates were among the elder and young children and the highest incidence of deaths in greater than 65 years of age. Among these cases of hospitalization, gastroenteritis caused nearly 61% of the cases [11]. Human body contains normal microbial flora which lives in a symbiotic relationship with the body. They derive nutrients for their survival and also protect our body from invading pathogens. Although this normal flora protects the body from pathogens, they also tend to cause diseases. Especially the organisms living in a populated ecosystem of the gastrointestinal tract, these bacteria invade the intestinal cells and cause diseases [12]. This normal microbial flora of human body may turn out to be pathogens under certain predisposing factors like genetics, age, delivery pattern, diet and antibiotics. [13].

Antibiotics that declare to inhibit the growth of the pathogens, also destroys the normal microbial flora which acts like a two edged weapon. Due to continuous uptake of antibiotics, the essential commensal flora is destroyed while the undesirable pathogens overgrow them. This disruption in the microbial community facilitates the growth of the pathogens [14]. Hence an alternate therapy to antibiotic is an essential solution to treat the pathogens but also acts as a probiotic to the intestinal ecosystem. Siddha, traditional Indian system of medicine has a potential role as an antibiotic and as a probiotic too. Siddha has a number of single herb and polyherbal formulations of plants, herbs, minerals and animal origin. Many siddha preparations have been proved to be antimicrobial [15] (16). The study on the antimicrobial activity of medicinal plants and folk medicines focussed majorly on the phytochemicals present in

their isolated compounds such as alkaloids, flavonoids, sesquiterpene lactones, diterpenes, triterpenes or naphtoquinones [17] [18]. In the present study siddha medicines that are commonly prescribed for enteric diseases were chosen and named as G1, G2, G3, G4, G5, G6, G7, G8, G9, G10. These medicines were screened for their antimicrobial activity against seven pathogens *E.coli, Salmonella, Shigella flexneri, Clostridium perfringens, Helicobacter pylori, Staphylococcus aureus* and *Streptococcus pyogenes.* The antimicrobial screening was carried out by agar well diffusion method and their Minimum Inhibitory Concentration was measured using the broth dilution method [19]. From the test results it was found that G1 has a potential antimicrobial activity against six of the enteric pathogens tested while G2 and G9 was found to have significant activity against *Staphylococcus* and *Shigella* species. G9 was active against *Clostridium perfringens.* The Minimum Inhibitory Concentration for the drugs G1, G2, G3 and G9 were also determined through this study. Thereby it is concluded that G1, G2, G4 and G9 act as a potential antimicrobial agents against enteric pathogens.

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REFERENCES

1. Glynis Kolling, Martin Wu and Richard L. Guerrant. (2012). "Enteric pathogens through life stages". *Frontiers in Cellular and Infection Microbiology*. doi: 10.3389/fcimb.2012.00114
2. World Health Statistics (2009). World Health Organization https://www.who.int/gho/publications/world_health_statistics/EN_WHS09_Full.pdf
3. Mun Fai Loke, Aimi Hanafi. (2019). "Molecular Mechanisms Responsible for Drug Resistance". *Encyclopedia of Bioinformatics and Computational Biology*. Vol. (3) 926-931.
4. M. J. Wallace, S. R. S. Fishbein and G. Dantas. (2020). "Antimicrobial Resistance in enteric bacteria: current state and next-generation solutions". *Gut Microbes*. 12 (1): 1799654 doi: 10.1080/19490976.2020.1799654.
5. Satyabrata Bag, Tarini Shankar Ghosh, Sayantan Banerjee, Ojasvi Mehta, Jyoti Verma, Mayanka Dayal, Anbumani Desigamani, Pawan Kumar, Bipasa Saha, Saurabh Kedia, Vineet Ahuja, Thandavarayan Ramamurthy, Bhabatosh Das. (2019). "Molecular Insights into Antimicrobial Resistance Traits of Commensal Human Gut Microbiota". *Microb. Ecol.* 77(2):546-557. doi: 10.1007/s00248-018-1228-7
6. Pawan Kumar, Satyabrata Bag, Tarini Shankar Ghosh, Prasanta Dey, Mayanka Dayal, Bipasa Saha, Jyoti Verma, Archana Pant, Shruti Saxena, Anbumani Desigamani, Preety Rana, Dhirendra Kumar, Naresh C Sharma, Pranita Hanpude, Tushar K Maiti, Asish K Mukhopadhyay, Rupak K Bhadra, G Balakrish Nair, Thandavarayan Ramamurthy, Bhabatosh Das. (2017). "Molecular Insights into Antimicrobial Resistance Traits of Multidrug Resistant Enteric Pathogens isolated from India". *Sci Rep*. Oct 31; 7(1):14468. doi: 10.1038/s41598-017-14791-1.
7. Acharya Balkrishna, Pallavi Thakur and Anurag Varshney. (2020). Phytochemical Profile, Pharmacological Attributes and Medicinal Properties of Convolvulus prostrates- A cognitive enhancer herb for the Management of Neurodegenerative Etiologies. *Front. Pharmacol*., 11(171): 1-12.
8. Rajesh Dabur, Amita Gupta, T K Mandal, Desh Deepak Singh, Vivek Bajpai, A M Gurav, G S Lavekar. (2007). "Antimicrobial Activity of Some Indian Medicinal Plants". *Afr. J. Trad. CAM* (2007) 4 (3): 313 – 318
9. Mounyr Balouiri, Moulay Sadiki, Saad Koraichi Ibnsouda. (2016). "Methods for in vitro evaluating antimicrobial activity: A review". *Journal of Pharmaceutical Analysis*. 6 (2016): 71–79
10. Irith Wiegand, Kai Hilpert, Robert E W Hancock. (2008). "Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances". *Nature Protocols*. vol. 3: 163–175
11. R T Trevejo, J G Courtney, M Starr, D J Vugia. (2003). "Epidemiology of salmonellosis in California, 1990-1999: morbidity, mortality, and hospitalization costs". *Am J Epidemiol*. 157(1): 48-57. doi: 10.1093/aje/kwf155

12. Charles Patrick Davis. (1996). "Normal Flora. Medical Microbiology". 4th edition. Chapter 6 Galveston (TX): University of Texas Medical Branch at Galveston.
13. Nihal Hasan and Hongyi Yang. (2019). "Factors affecting the composition of the gut microbiota, and its modulation". *Peer J*. 7: e7502. doi: 10.7717/peerj.7502
14. Nathan J. Klingensmith and Craig M. Coopersmith. "The Gut as the Motor of Multiple Organ Dysfunction in Critical Illness". *Crit Care Clin*. 2016 Apr; 32(2): 203–212. doi: 10.1016/j.ccc.2015.11.004
15. Srinivasan D., Sangeetha Nathan, Suresh T., Lakshmana Perumalsamy P. (2000). "Antimicrobial activity of certain Indian Medicinal Plants used in folkloric medicine". *Journal of Ethnopharmacology*. 74(3). 217-220. doi.org/10.1016/S0378-8741(00)00345-7
16. Peter G. M., Peter A.A., Joyce O., Esther N. M., Christine C. B., (2013). "Antimicrobial Activity and Probable Mechanisms of Action of Medicinal Plants of Kenya: Withania somnifera, Warbugia ugandensis, Prunus africana and Plectrunthus barbatus". *PLoS ONE* 8(6): e65619. doi:10.1371/journal.pone.0065619
17. Rios J.L., Rechio M.C. (2005). "Medicinal plants and Antimicrobial Activity". *Journal of Ethnopharmacology*. 100(1-2). 80-84. doi.org/10.1016/j.jep.2005.04.025
18. Hassan SW, Umar RA, Lawal M, Bilbis LS, Muhammad BY, Dabai YU, *et al*. "Evaluation of antibacterial activity and phytochemical analysis of root extract of *Boscia angustifolia*". *Afr J Biotechnol 2006*; 5(18) 602-7.
19. K. R. Aneja. "Experiments in Microbiology, Plant Pathology and Biotechnology". *New Age Publishers*, 2005, p. 69, p. 390

Table 1. List of Siddha Medicines, their ingredients, dosage form and Indications

S.NO.	NAME OF THE MEDICINE	INGREDIENTS	DOSAGE FORM	INDICATIONS
G1	Elathy Chooranam	Elettaria cardamomum, Cuminum cyminum, Syzygium aromaticum, Glycyrrhiza glabra, Emblica officinalis, Cinnamomum tamala, Cinnamomum verum, Murraya koenigi, Santalum album, Nardostachys jatamansi, Foeniculam vulgare, Saccharum officinarum	Powder	Burning micturition, biliousness, scaling of urine
G2	Gunmakudori Mezhugu	Sodium chloride impure, Sodium chloride, Sodium biborate, Sodium bicarbonate, ammoni chloridum, potassium nitrate, Zingiber officinale, Piper longum, Piper nigrum, Carum copticum, Syzygium aromaticum, Costus speciosus, Ferula asafetida, Allium sativum, Palm jiggery, honey	Paste	Peptic ulcer, Sluggish digestion, Indigestion
G3	Athimathura Chooranam	Glycyrrhiza glabra	Powder	Stomach ailments
G4	Sangu Parpam	Tribune pyrum, Pistia stratiotes	Powder	Gastritis, peptic ulcer, colic, other ailments of stomach
G5	Kukkil Parpam	Shorea robusta	Powder	Burning micturition, Anuria, dysentery, Uro-genital disorders

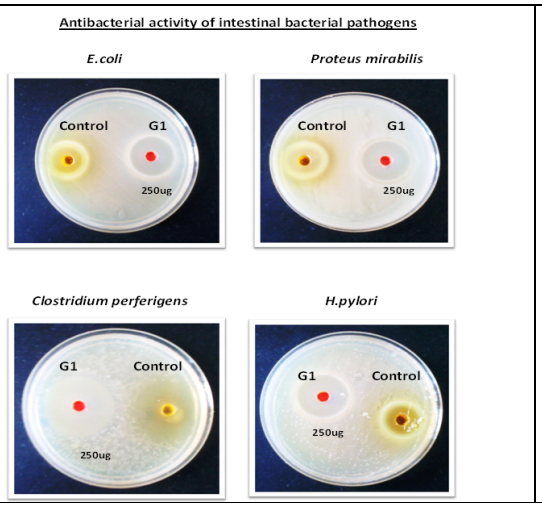
G6	Silasathu Parpam	Calcium sulphate dehydrate	Powder	Burning micturition, retention of urine, inflammation of urethra
G7	Venpoosani Legium	Saccharum officinarum, Cuminum cyminum, Coriandrum sativum, Saussurea lappa, Elattaria cardamomum, Myristica fragrans, Piper nigrum, Quercus infectoria oliver, Glycyrrhiza glabra, Abies spectabilis, Apis mellifera, Bos indicus Cucurbita maxima, Pandanus tectorius, Cocus nucifera, Asparagus racemosus, Citrus aurantifolia	Legium	Dysuria, painful micturition, inflammations in the urogenital tract
G8	Thaleesathi Chooranam	Taxus baccata, Cinnamomum zeylanicum, Eletteria cardamomum, Zingiber officinale, Glycyrrhiza glabra, Ferula foetida, Emblica officinalis, Saussurea lappa, Piper longum, Cumimum cyminum, Anethum sowa, Nigella sativa, Piper longum, Syzygium aromaticum, Myristica fragrans, Piper nigrum, Nardostachys jatamansi, Cinnamomum verum, Michelia champaca, Embelia ribes, Trachyspermum ammi, Coriandrum sativum, Saccharum officinarum	Powder	Abdominal pain, Burning sensation of stomach, Burning micturition
G9	Thriphala Chooranam	Emblica officinalis, Terminalia chebula, Terminalia bellirica	Powder	Constipation, ulcer
G10	Thriphala Karpam	Terminalia chebula, Emblica officinalis, Terminalia bellirica, Acacia catechu, Pterocarpus marsupium	Powder	Peptic ulcer, urinary calculi

Table 2. Zone of inhibition in mm at 250µg/ml

S.no	Name of the pathogen	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	Control
		Minimum zone of inhibition in mm 250µgm										
1.	*E. coli*	10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	15
2.	*P. mirabilis*	12	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	15
3.	*H. pylori*	10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	15
4.	*S. flexneri*	13	6	Nil	Nil	Nil	Nil	Nil	Nil	7	Nil	15
5.	*S. aureus*	12	4	Nil	Nil	Nil	Nil	Nil	Nil	8	Nil	15
6.	*Strep. pyogenes*	Nil	Nil	Nil	7	Nil	Nil	Nil	Nil	Nil	Nil	15
7.	*Clostridium perfringens*	10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	7	Nil	15

Table 3. Determination of Minimum Inhibitory Concentration (MIC)

S.no	Name of the pathogen	G1	G2	G4	G9
		Minimum inhibitory concentration(MIC)			
1.	*E. coli*	100	Nil	Nil	Nil
2.	*P. mirabilis*	75	Nil	Nil	Nil
3.	*H. pylori*	85	Nil	Nil	Nil
4.	*Shigella flexneri*	50	150	Nil	130
5.	*Staphylococcus aureus*	75	175	Nil	125
6.	*Strep. pyogenes*	Nil	Nil	140	Nil
7.	*Clostridium perfringens*	100	Nil	Nil	125



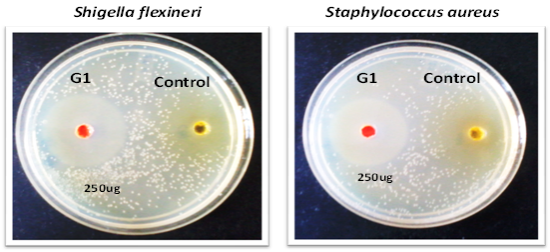


Fig. Zones of Inhibition of G1

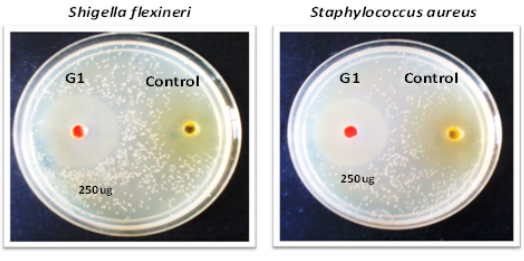


Fig. 2 Zones of Inhibition of G4

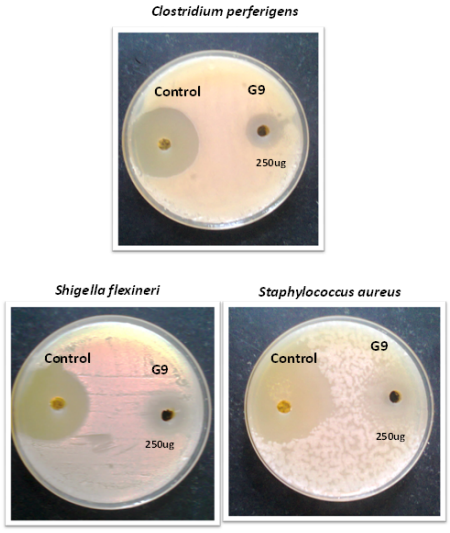


Fig. 3 Zones of Inhibition of G9

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RESEARCH ARTICLE

Impact of Digital Financial Inclusion during COVID-19 Pandemic

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ABSTRACT

This new decade has seen the world affected by an unprecedented health predicament with a pandemic that has been never ever been experienced earlier in our lifetimes, so ruthlessly affecting numerous individuals, families and a huge section of the community. Nearly many people have been affected by COVID-19 (Corona Virus Disease). Most of the employees are facing restrictions in their jobs at their work places. Therefore, as a productive outcome of the outbreak, state governments are bound to provide stimulus packages to people who are unable to afford feeding their families. Mostly, banks are affected by the pandemic since only half percent of the employees are allowed to work in the branches. The opening and closure hours of the banks have been restricted in an effort to control the disease. Most of the employees are requested to work remotely from their homes with an intention to curb the spread of the disease.

Keywords: Digital, Finance, Employees, Banks, Disease, Pandemic, Financial, Individuals, Small-scale, Inclusion

INTRODUCTION

Much is being said about digital change in the banking process and much money invested, but this has not brought the expected results. Some grave reasons such as lack of proper planning and deficiency in senior executive support may be attributed to the difficulty that some banks face in this regard. This has been substantiated by Aurelie L'Hostis, author of the Forrester report which states "While a handful of banks are pushing ahead with their digital transformation, others are still struggling to create and execute a coherent transformation strategy."However, neobanks and fintechs have started throwing light on how to do bring about this digital transformation, how to do it the right way and effectively. Digital transformation, now considered one of the most popular buzz phrases in banking, is indeed the trend today, at least with consumers. Financial inclusion is a process of offering financial and banking services to individuals. It is the availability of opportunities to acquire financial services. It is a method by

which individuals and corporate businesses can obtain in a timely manner, relevant and nominal financial products and services regardless of their income and savings. Financial inclusion helps the poor and marginalised people to make use of their money in the best way possible. This would be one of the positive ways to involve society and make the majority participate in financial management judiciously. It benefits individuals, society and economy as a whole. The Covid-19 pandemic indicates that the inclination towards greater digitalization of financial services will stay here. Digital financial inclusion certainly saves operating costs, brings greater efficiency and generates greater profitability.

Statement of the Problem

The Covid-19 pandemic has resulted in critical and prolonged disruptions affecting individuals and small-scale firms. Small firms and under-privileged households can benefit from online banking, mobile banking services and fintech services. Financial inclusion is a digital financial service that increases economic growth. Due to the limited bank hours, there are restrictions in procedures of banks that the household and society have to follow. As the pandemic is bound to increase, the use of these kind of financial services has put forth challenges to individuals and industries alike. It has also served to spotlight dissimilar access to digital infrastructure. The restrictions have affected not only society but also banks in an effort to curb the growth and increase of Corona virus. With more restrictions that are being coerced at the moment, access to finance becomes crucial in today's digital environment. This crucial situation, therefore, cries out for the dire need to provide financial solutions to the underprivileged.

Objectives of the Study

To understand the economic crisis being experienced by individuals, small scale industries and society in general.
To suggest some tentative solutions for the existing problem.

Need for the Study

The highly complicated global scenario today urgently calls for an in-depth study into the problem with a motive to unearth a solution that would be a boon to the economically underprivileged. This would include mode of savings and loan services that would not be burdensome or painful to the client. It is not an unknown fact that about 30 years ago banking systems dealt with a lot of paperwork. Computers and the internet had not advanced enough then. But overnight much transformation has taken place resulting in a huge push to go digital that came as a repercussion of the pandemic. This mega change converted the shape of the entire banking industry by making it go digital. Digital banking implies converting all traditional banking systems to online or digital mode. Bank services could include deposits, transfers, withdrawals, as well as applying for various financial services, account handling, loan management, and bill payments. Digital banking removes the need for paperwork such as demand drafts, cashing cheques, or pay-in slips. In this, one has the liberty to perform all banking activities 24/7 without literally going to the bank. On the other hand, digital banking facilities are accessible with internet connection and electronic gadgets like mobiles, laptops, or tabs.

Digital banking helps the clients to overcome the hassles of issuing demand drafts or cheques. For example, it is easy to transfer money from one account to another without a visit to the bank. Thus, transactions can be made anytime anywhere. This therefore guarantees a low risk of the viral infection and ensures safety. Some very popular online money transfers are IMPS (Immediate Payment Service), RTGS (Real-Time Gross Settlement), and NEFT (National Electronic Fund Transfer) and quite encouragingly, a majority of the population have started enjoying these digital facilities. Besides, one has the benefit of downloading e-bank statements at any point of time. Mostly, all the bank statements can be stored on mobile phones or laptops for one's convenience to be accessed easily. This spares them from visiting banks and taking printed copies of statements, thereby preventing unwanted contact during the pandemic times. Moreover, the installation of ATM machines in every nook and corner is a great boon to people to withdraw cash, at any time of the day or night. Digital banking paves way for a smooth payment of bills by simply logging in to an individual's bank account. All kinds of bills such as electricity, phone, gas, and television can be paid

conveniently via digital banking. Furthermore, one can open a fixed deposit account in an instant, invest in mutual funds, and apply for loans and various insurance policies as well.

Increasing usage of smartphones and the availability of strong network connectivity have resulted in making mobile banking systems a blessing. Through smartphones one can easily download apps for transaction purposes. Google Pay, Apple Pay, BHIM, SBI's Yono, Payzapp, beside several others, are gaining popularity amidst the COVID-19 outbreak. By means of scanning a QR code or knowing the contact number of the beneficiary, one can conveniently enter into a transaction. All banking services therefore, are at one's doorstep through the mobile banking apps. Going the digital way helps one to track credits and debits of the account as banks send SMS or e-mails of transactions. These notifications safeguard one from frauds and even otherwise such cases can be brought immediately to the notice of the bank authorities. Digital banking also displays transaction history and any pending payments as well.

The Future of Digital Banking

Today this topic seems relevant as digital banking sector will continue to grow in the forthcoming years with a few changes in technology. Several banks are already utilizing artificial intelligence to meet the financial demands and expectations of customers. Today while it is artificial intelligence, tomorrow it may be something else that may augment digital banking to greater heights.

Scope of the Study

The study will provide an opportunity to understand the requirements of the people and the psychological apprehensions they harbour before approaching the banks and other financial services while applying for loans etc. It is the responsibility of such financial service providers to remove such qualms and uncertainties through proper counselling and encouragement thereby building up the consumers' confidence to apply for loans and small-time savings that would come to their instant aid even if the pandemic were to persist.

RESEARCH METHODOLOGY

Nature of Research

Descriptive research is employed in this study. It provides answers to such questions as who, what, where and how.

Sampling Framework

A sample framework has been designed from a sector of the population available for consideration. So Convenient Sampling is taken for study.

Data Collection

Secondary data has been collected from various journals, newspapers, magazines, internet and respondents at random.

Financial Inclusion and Digital Finance

The foundation for interconnection between financial inclusion and digital finance is the premise that a large number of population own mobile phones and related devices that can improve access to finance. Individuals and householders should be able to afford internet connectivity as digital finance is envisaged to have positive effects of financial inclusion. There is a positive correlation between the utilization of digital finance and its approach to financial services.

Challenges faced by individuals and Society during Covid times

The DFS(Digital Finance Services) uses enterprising marketing strategies to induce the low-level individuals and householders to use various digital platforms thereby leading to lower digital financial inclusion for those with poor

income because the monetary pay off to the digital service providers will be higher when compared to middle and higher income groups. Digital financial inclusion compels individuals, small scale industries and normal bank account holders to activate the bank related transactions digitally to improve the welfare of bank account holders but it does not improve and benefit the individuals if they do not have a bank account. Digital finance services will be of no use to individuals who do not use digital devices for financial transactions. In this regard the main problems faced are:

- Digital financial inclusion mainly relies on internet connectivity
- Some digital platforms are fee-based which will benefit only the high and medium sector people, but where poor individuals are concerned, they will not be able to afford the transaction costs
- The individuals who do not have devices like mobile phones, etc., will not gain access to digital financial transactions

Financial inclusion & Covid-19 recovery

Due to the pandemic, lockdowns have led to long waiting times for those who have opted for telephone banking because many branches of the banks have either merged or been forced to close down. So, the individuals of all ages experience the benefits of digital banking with the help of modern technological tools. The impact of digital transformation on financial services has been realized over the past few years. This is through the shift from traditional banking to online and mobile banking digital services that include digital payments. Hence, digital financial inclusion plays a vital role in attracting the investors, individuals and the society. It can also increase the growth of gross domestic products and may bring down the poverty level of the country.

Suggestions

From the pandemic point of view, digital financial inclusion could be conveniently accessed by the low-income individuals, provided the transaction fee is reduced. If the digital service providers could dispense with fees, or waiver the transaction amount, then digital financial services would flourish, hopefully. The regulations and procedures could be altered accordingly to suit the needs of the people. It could increase the transaction limits and maximize the wallet balance. It could also lower the minimum amount required for a transaction and that would certainly enhance the digitalization of routine payments. World Bank states that 40% of adults in low-income countries do not have a formal means of identification, which makes it difficult for them to access financial services. It is here that Know-Your-Customer (KYC) regulations, an essential component to maintain the integrity of a financial system, can have positive consequences. As a result, the lockdown measures and the risks associated with cash have made it mandatory for many central banks to reassess their KYC requirements.

Thanks to the rising popularity of the mobile and the internet, underbanked sections of society can also get the benefit of financial services from the comfort of their homes. Digitization of loan application processes plays a vital role in helping borrowers to apply for loans wherever they may be. This is a very important prerequisite in a post-pandemic world. Digital lending platforms reduce the dependence on formal financial documents like tax returns, bank statements etc. as well as face-to-face customer dealings. On the other hand, highly developed technologies such as Artificial Intelligence (AI), Machine Learning (ML), video KYC, Aadhar-based KYC, account aggregators, assist lenders to easily access customer data legally, and ensure appreciable services. As banks have to prioritize their focus mostly on standard parameters like salary, credit scores, etc., fintech lenders can experiment with a more innovative approach by collecting data such as geographical pin codes to specify acutely affected COVID zones, employment industry to check which of the industries are more affected comparatively, and also the employment status of potential borrowers, employment proof recheck regarding layoffs and salary adjustments, and such relevant information. Thus, by adopting digital technologies, fintech lenders can help people get easy access to capital, create a faster, safer, and more efficient financial system, which will protect the interests of both the borrowers and the lenders. Thus, India's promising digital lending ecosystem has the potential to boost up the country's economic progress by being both drivers and enablers of financial inclusion.

CONCLUSION

With the pandemic changing the way of thinking with regard to financial services, it is time for investors and governments to think holistically and on a long-term basis about this new reality and compromise with the hither to challenging circumstances. Financial inclusion and digital financial services can be crucial tools in improving levels of financial inclusion. It would identify issues of inequality and potentially help to minimise the gap between the developing countries and the developed nations. COVID-19 has impacted financial inclusion trends across the world in many complicated ways. The shift towards digital financial services had come to the aid of the public even before the pandemic started. Thus, many low-income households and small business firms had been much benefitted. Today lockdowns and social distancing are accelerating the demand for use of digital financial services. In fact, in some countries digital payment services have started offering digital lending. Marketplace lending uses digital platforms to directly connect lenders to borrowers, even on a small scale, thus creating a confidence in individuals and small-time traders. However, the pandemic shows that the inclination towards digitalization of financial services has come to stay. So, to infuse a greater trust in the consumers there is a dire need to address the inequalities during and after the ongoing crisis. This can only take place by striking a right balance between enabling financial innovation and addressing several other risks, such as insufficient consumer protection, lack of financial and digital literacy, poor access to digital infrastructure, and data bases that demand action at the national level. The aim of this study, therefore, is to illustrate the opportunities associated with the use of digital financial services, thereby providing ideas on how to understand the changes affecting the overall approach and perspectives with a view to promote financial inclusion and development.

REFERENCES

1. https://www.internationaltaxreview.com/article/b1mph5b8yvgwwv/how-covid-19-has-impacted-the-financial-services-sector
2. http://www.worldbank.org/en/news/feature/2014/04/10/digital-finance-empowering-poor-new-technologies
3. https://www.sciencedirect.com/science/article/pii/S2214845017301503#bib36
4. T.Beck & M.Brown: Use of banking services in emerging markets-household-level evidence. CEPR Discussion Papers 8475. CEPR Discussion Papers(2011)
5. https://investmentmonitor.ai/global/will-the-covid-crisis-improve-financial-inclusion
6. https://thefinancialbrand.com/108484/coronavirus-digital-banking-transformation
7. http://www.fao.org/3/cb2109en/CB2109EN.pdf

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REVIEW ARTICLE

Anti-Oxidant *(*Kayakarpam*)* Property of Kadukkai *(Terminalia chebula)* in Siddha System – A Review

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ABSTRACT

Anti-oxidants play a vital role in preventing the diseases by reducing the oxidative stress and help in reducing the various Non-Communicable Diseases (NCDs) like cardiovascular diseases, cancer, obesity, diabetes, hypertension, chronic lung diseases and infertility etc. 41 million people were died in every year because of NCDs. 71% of all deaths globally are due to NCDs. In Indian traditional medicine system particularly in Siddha, the anti-oxidant is termed as *Kayakarpam* / rejuvenation. In antient time, Siddhars had practised *Kayakarpa* therapy for prevention of diseases and aging. They were listed some medicinal herbs and herbal formulations as *kayakarpa* medicine. *Kadukkai (Terminalia chebula)* is one among them. The present study is aimed to review the anti-oxidant property and importance of *Kadukkai* in *kayakarpa* therapy. *Kadukkai (Terminalia chebula)* is indicated for various non-Communicable diseases (NCDs) like cardiovascular diseases, hypertension, diabetes mellitus, infertility etc. Siddhars advised to consume *Kadukkai* as anti-oxidant in all season's monsoon, autumn, early winter, late winter, spring and summer with suitable *anubanam*(vehicle) *Induppu*(rock salt), *Sarkkarai*(sugar), C*hukku* (dried ginger),*Thippili (long pepper),Thean* (honey) and *Vellam* (jaggery) respectively. This *Kadukkai karpam* is the only *kayakarpa* medicine, recommended for all seasons in a year to enhance the general immunity, improve the health status, prevention of non-Communicable diseases (NCDs) and seasonal conditions like cough, cold, fever, constipation, abdominal discomfort, urinary tract infections, etc. Moreover, the drug *Kadukkai (Terminalia chebula)* was scientifically proved for its anti-oxidant property and *Kadukkai* is one of the important ingredients in many more anti-oxidant siddha formulations. This review study is

recommended that *Kadukkai (Terminalia chebula)* can be used as potent anti-oxidant agent for the prevention and management of non-communicable diseases (NCDs).

Keywords: *Kadukkai, Terminalia chebula*, Anti-oxidant, Siddha medicine

INTRODUCTION

Non communicable diseases (NCDs) also called as chronic diseases or lifestyle-related diseases. NCDs are of long duration and also slow progression. The four major categories of NCDs are cardiovascular diseases, cancers, diabetes and chronic respiratory diseases [1]. Prevalence of NCDs in India is 116 per 1,000 populations, with hypertension, digestive diseases and diabetes leading the burden, a survey report by trade association Assocham showed. The report found that more than 2/3of individuals suffering from NCDs are in the most productive-life age groups between 26 and 59 years. Now WHO focused on prevention and control of NCDs, because long term medication used for NCDs are leads to so many complications. Oxidative stress is the main reason for the progression of NCDs. Oxidative stress which is damage to the both nuclear and mitochondrial DNA has detrimental effects, leading to uncontrolled cell proliferations or accelerated cell deaths [2]. Physical inactivity, unhealthy diets like less intake of fruit, vegetables, and whole grains, but high intake of salt and fat and habits like tobacco use through smoking, second and smoke, and smokeless tobacco usage and the harmful use of alcohol are the main behavioural risk factors for NCDs. Even though morbidity and mortality from NCDs mainly occur in adulthood, exposure to risk factors begins in early life. In India, nearly 5.8 million people die due to NCDs in every year or in other words 1 in 4 Indians has a risk of dying from NCD before they reach the age of 70[3].

However, we living in modern societies has proven to be a double-edged sword, with several elements of the western lifestyle actually contributing to the development of age-related chronic diseases, in particular in NCDs such as cardiovascular diseases, diabetes, neurodegenerative dementia and cancer [4]. Thus, Anti-oxidants play a vital role in preventing the diseases and help in reducing the various NCDs. These are the compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction. The adverse effects of synthetic antioxidants, researchers and scientific community have channelled their interest in isolating natural antioxidants [5]. Antioxidants takes place naturally and plays an important role in process of aging. It prevents the free radical induced tissue damages by preventing the formation as well as accumulation of radicals, scavenging them, or by promoting their decomposition and impede the progress of chronic diseases [6]. Siddha system is one of the ancient traditional medical systems in India. Siddhars treated the various health ailments with natural resources of herbal, metals, minerals, zoological and marine products. In Siddha system, a unique science called *Kayakarpam,* an elixir promoting the process of rejuvenation. Rejuvenation helps to increase the longevity of human life and prevent aging, greying of hair, wrinkling and even death. Siddhars used the *Kayakarpam* for the long living through preventing the diseases and treating the acquired diseases. For this purpose, Siddhars listed some medicinal herbs and herbal, herbo-mineral formulations as *kayakarpa* medicine. Some herbs used as antioxidants in Siddha such as Ginger (*Zingiber officinale*), Ashwagantha (*Withania somnifera*), Katralai (*Aloe barbadensis*), Karisalai (*Eclipta prostrata*), Vallarai (*Centella asiatica*), Nelli (*Phyllanthus emblica*), Thulsi (*Ocimum sanctum*), Lemon (*Citrus limon*). *Kayakarpam* may be single medicinal herb or a preparatory medicine that should be taken for a specific period of time [7].

Kadukkai *(Terminalia chebula)* is one of special *Kayakarpa* medicine which is being used tremendously by traditional vaithiyars and siddha practitioner. The present study is aimed to review the anti-oxidant property and importance of Kadukkai in *kayakarpa* therapy from various ancient siddha literatures and scientific journals. Kadukkai *(Terminalia chebula)* is indicated for various NCDs like cardiovascular diseases, hypertension, diabetes mellitus, infertility etc. *Kadukkai* is the only herb which is being used tremendously in siddha and also advised to consume *Kadukkai* as anti-oxidant in all seasons with suitable vehicle. In our system *anubanam* (vehicle) which plays an important role in the

treatment of diseases. Our literatures showed that one herb or medicine with different vehicle is indicated for different types of diseases. In this review study is recommended that *Kadukkai (Terminalia chebula)* can be used as potent anti-oxidant agent for the prevention and management of NCDs.

Kadukkai *(Terminalia chebula)*

Kadukkai (*Terminalia chebula)*is a plant species belonging to the genous *Terminalia*, family Combretaceae and widely distributed in India, Srilanka, kumaon and Burma upto an altitude of 1500 (-2000)m. *Terminalia chebula*. It is a flowering evergreen tree called in English the black myrobalan. It is also known as Haritaki (Sanskrit and Bengali), Harad (Hindi), Karkchettu (Telugu), Kadukkai (Tamil), Harada (Marathi & Gujrati) [8]. It's also known as "King of medicine" in Tibet [9]. It acts as astomachic, effective purgative (gentle laxative), astringent and alterative, which is indicated for Jaundice, obesity, ageusia, hypertension, eye diseases, dropsy, pepticulcer, aneamia, vomiting, fistula, stomatitis, hydrocele, fever, cough, asthma, urinary diseases, piles, worm infestations, rheumatism, scorpion sting. As well as it gives strength to seven body constituents and prevents aging [10].

Kadukkai karpam

In Siddha text *Gunapadam mooligaivaguppu* [11] quotes that *Terminalia chebula* "Cares the patients as a mother cares a child" and also superior to the nourishing mother for its extraordinary healing power. It possesses antioxidant and immunomodulatory effect. *Terminalia chebula* possess Astringent, Pungent, Bitter, Sweet and Sour taste. These tastes help to reduce or balance the vitiated *vatham, pitham* and *kabham* (*Thirithodam*) and also it has hot potency [12]. This *Kadukkai karpam* is the only *kayakarpa* medicine, recommended for all seasons in a year to enhance the general immunity, improve the health status, prevention of NCDs and seasonal conditions like cough, cold, fever, constipation, abdominal discomfort, urinary tract infections, etc. As *kayakarpam* therapy, Kadukkai *(Terminalia chebula)* can be administered with different types ofvehicle*(anupanam)* based on seasonal variations for enhance as well as strengthen our immune system which is mentioned in Siddha literature[11] and tabulated in Table no: 1

Phytochemicals

Mariappan Amutha *et al,* conducted a study on Phytochemical analysis of *T. Chebula.* Through the results the quantity of alkaloids (2.85mg/g), phenols (1.72 mg/g), terpenoids (1.06 mg/g) and flavonoids (0.560 mg/g). Among the flavonoids, quercetin (0.2336 mg/g) was found huge amount when compared to rutin (0.017 mg/g) and gallic acid (0.045mg/g) [13]. Neelam Kushwaha et al., studied that the Aqueous extract of *Terminalia chebula* showed presence of phenol, carbohydrate and glycosides whereas, negative for alkaloids, protein and saponin. Its ethanolic extract showed presence of phenol, protein, saponin, carbohydrate and glycosides and absence of alkaloids. The highest antioxidant function was found in the ethanolic extract of *Terminalia chebula* [14]. Chia Lin Chang and Che San Lin carried out a studyon Phytochemical Composition, Antioxidant Activity, and Neuroprotective Effect of *Terminalia chebula* Retz Extracts. The results revealed that the total phenolic content of three extracts varied from 867.2 to 1041.8 mg gallic acid/g extract. The total tannin content of the three extracts varied from 33.9 to 40.3%mg extract [15]. Kumar KJ studied that *Terminalia chebula* contains several phytoconstituents like tannins, flavonoids, sterols, amino acids, fructose, resin and fixed oils *etc*., and it was rich in different tannins(approximately 32% tannin content). Further he stated that the content of tannin islargely depends on its geographiclocation [16].

Juang *et al*., isolated 14 hydrolysable tannins names gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulegic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl-b-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose and terchebulin from *T. chebula* fruit [17]. Trease and Evans pharmacognosy stated that *Terminalia chebula* contains about 20-40% of tannins [18].

Anti-oxidant activity

G.P. Senthilkumar *et al.,* reported that the Evaluation of Antioxidant Potential of *Terminalia chebula* fruits studied instreptozotocin-induced diabetic Rats. The study results revealed that the changes in the level of vitamin C in the plasma of all the experimental rats. The level of vitamin C decreased in streptozotocin-induced diabetic rats. The

presence of biologically active ingredients in the fruit extract may be responsible for the antioxidant properties of the *T.chebula* fruits, which in turn may be partially responsible for its antidiabetogenic properties [19]. Hazra B *et al.,* carried out a study to evaluate the *in- vitro* antioxidant and reactive oxygen species scavenging activities of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis* fruit extracts. The 70% methanol extracts for in-vitro total antioxidant activity along with phenolic and flavonoid contents and reducing power and scavenging ability of the extracts for radicals were studied. It exhibiting their antioxidative properties and flavonoid content follow the order *T. chebula*>*E. officinalis*>*T. belerica* and this order also for superoxide and nitric oxide radicals [20]. Sarmisthasaha *et al.,* studied that the Antioxidant activity of polyphenolic extract of *Terminalia chebula* Retz fruits. In this study the total phenolic content of the polyphenolic extract of *T. chebula* was significantly correlated with its total antioxidant capacity (R = 0.992, $p < 0.05$), DPPH radical scavenging activity (R = 0.971, $p < 0.05$), nitric oxide radical quenching activity (R = 0.995, $p < 0.05$) and hydrogen peroxide scavenging activity (R = 0.990, $p < 0.05$). This result indicated that the phenolic contents of *T. chebula* were responsible for its antioxidant activity [21]. Chen X *et al.,* done an excellent anti-oxidant study on *Terminalia chebula*. 6 extracts and 4 pure compounds of *Terminalia chebula* exhibited the *in-vitro* antioxidant properties through anti-lipid peroxidation, anti-superoxide radical formation and DPPH activities at different concentration. The results demonstrated that tri-ethyl-chebulate was a strong antioxidant and free-radical scavenger, which might contribute to the anti-oxidative ability of *Terminalia chebula* [22].

Saleem et al.,reported that the *T.chebula* has a stronger antioxidant activity than alpha-tocopherol and HPLC analysis with diode array detection indicated the presence of hydroxybenzoic acid derivatives, hydroxycinnamic acid derivatives, flavanol aglycones and their glycosides, as main phenolic compounds in *T.chebula* [23]. Hyun-Sun LEE *et al.,* evaluated the protective effects of an aqueous extract of fruit of *T. chebula* on the *tert*-butyl hydroperoxide (*t*-BHP)-induced oxidative injury observed in cultured rat primary hepatocytes and rat liver. *T. chebula* fruits had potent antioxidative and protective effects against *in-vitro* free radical generation and *t*-BHP-induced oxidative hepatotoxicity in rat primary cultured hepatocytes and rat liver [24]. Karel D. Klika *et al.,* studied that the structural and conformational analyses and antioxidant activities of chebulinic acid and its thrice-hydrolyzed derivative, 2, 4-chebuloyl-β-D-glucopyranoside, isolated from the fruit of *Terminalia chebula*. The isolated compounds, chebulinic acid showed the highest radical scavenging activity in the DPPH assay. For the methyl linoleate assay, galloyl-free chebulinic acid and gallic acids, ethyl gallate, and luteolin all exhibited strong antioxidant activities whereas the activities of chebulinic acid and ellagic acid were only moderate. The fruit extract itself was most effective in both tests [25]. Praveen Kumar Vemuri et al, studied the phytochemical analysis and biochemical characterization of *Terminalia chebula* extracts for its medicinal use, this study revealed that Methonolic extract of *T.chebula* at 517nm exhibited maximum activity with 82% of free radical scavenging property [26].

Harpreet Walia *et al.,* conducted a study on Comparative analysis of antioxidant and phenolic content of chloroform extract/fraction of *Terminalia chebula*, in this study two chloroform extracts (CHL1&CHL 2) of *Terminalia chebula* fruit and prepared by maceration and sequential method respectively was compared for their antioxidant efficacy and phenolic content. The study results showed that CHL 2 extract of *T. chebula* prepared by sequential method exhibited good hydrogen donating, radical scavenging, metal chelating, reducing and antioxidant potential [27]. Mahesh R et al., stated that the *Terminalia chebula* in a polyherbal formulation (Aller-7/ NR-A2) inhibited free radical induced hemolysis and significantly inhibited nitric oxide release from lipopolysaccharide stimulated murine macrophages [28]. Lee HS *et al*, studied the protection of rat hepatocytes against oxidative toxicity by chebulic acid obtained from T. chebula Retz. It exhibited in vitro a free radical-scavenging activity and ferric-reducing antioxidant activity demonstrated that the treatment of hepatocytes with chebulic acid significantly reduced the tert-butyl hydroperoxide (t-BHP)-induced cell cytotoxicity, intracellular reactive oxygen species level, and the ratio of GSSH, oxidized form of glutathione (GSH) to the over total GSH (GSH + GSSG) (4.42%) as compared to that with t-BHP alone (8.33%) [29].

Chen X et al., studied that the Acetone extract of *T. chebula*has stronger antioxidant activity than alpha-tocopherol and HPLC analysis with diode array detection indicated the presence of hydroxybenzoic acid derivatives,

hydroxycinnamic acid derivatives, flavanol aglycones and their glycosides, as main phenolic compounds [30]. Rattanasena P. studied the Antioxidant and antibacterial activities of vegetables and fruits commonly consumed in Thailand. The results revealed that the extracts derived from *Terminalia chebula* Retz. when using distilled water and 80% ethanol as solvents were shown to have significantly high levels of DPPH scavenging (IC50 at 3.73+/-0.01 and 3.81+/-0.01 mg mL-1, respectively), FRAP antioxidant activities (80.85+/-0.10 and 65.93+/-0.11 mmol FeSO4 g-1of dry weight of fruit, respectively) and total phenolic compounds (13.10+/-0.06 and 10.66+/-0.02 mg gallic acid eq g-1 of dry weight of fruit, respectively) [31]. Zunlai Sheng *et al.,* studied that the Optimization of total phenolic content from *Terminalia chebula* Retz. fruits using standard methodology and evaluation of their antioxidant activities. The study results showed that compared to conventional solvent extraction (CSE), UAE extracts showed excellent DPPH radical, DPPH, ABTS scavenging activities and reducing power in a dose-dependent manner, and better than that of CSE extracts[32].

T. Vani *et al.,* carried out a study on the antioxidant properties of the Ayurvedic formulation *Triphala* and its constituents. The study results revealed that the Alcohol extracts of *Triphala* and its constituents were studied comparatively and found to be strong antioxidants[33]. Cheng HY *et al.,* studied that the Antioxidant and free radical scavenging activities of *Terminalia chebula*. The six extracts and 4 pure compounds of *Terminalia chebula* retz. were investigated for its anti-superoxide radical formation, anti-lipid peroxidation and free radical scavenging activities. The results showed that alltested extracts and pure compounds of *T. chebula* exhibited antioxidant property at different magnitudes of potency [34]. Mariappan Amutha et al., conducted a pilot study on the determination of antioxidant potential and lethal dosage of hydro alcoholic fruit extract of *Terminalia chebula.* The results revealed that the aqueous fruit extract of *Terminalia chebula* (AFETC) was found to scavenge free radicals effectively. The IC_{50}value of AFETC while scavenging DPPH radicals (37.4µg/ml), superoxide (37.85µg/ml) and nitric oxide (34.37µg/ml) were comparable to that of their corresponding reference compounds ascorbic acid (38.76µg/ml) and rutin (40.57µg/ml and 45.84µg/ml) [35]. Naik GH *et al.,* reported that the Aqueous extract of *T. chebula* inhibit xanthine/xanthine oxidase activity and was also an excellent scavenger of DPPH radicals. The results concluded that the aqueous extract of *T. chebula* acts as a potent antioxidant and it was able to protect cellular organelles from the radiation-induced damage, it may be considered as a probable radio protector [36].

Chia Lin Chang *et al.,* reported that the Phytochemical Composition, Antioxidant Activity and Neuroprotective Effect of *Terminalia chebula* Retz Extracts. The results revealed that the water extract appeared to have good antioxidant activities in cupric sulfate-Phen-Vc H_2O_2 and luminol-H_2O_2 assays. Pyrogallol-luminol assay showed the 95% ethanol extract to have good antioxidant activity. The methanol extract had the greatest total triterpenoid content and exhibited good antioxidant activity in the HRP-luminol-H2O2 assay [15]. Suchalatha, Subramaniyan et al., carried out a study on Antioxidant activity of ethanolic extract of *Terminalia chebula* fruit against isoproterenol-induced oxidative stress in rats. This study results showed that the significant antioxidant activity of the ethanolic extract of *Terminalia chebula* fruit could have scavenged the superoxide and hydroxyl radicals generated after myocardial ischemia and thus protects the myocardium injury [37].

CONCLUSION

Antioxidants have a positive effect on human health since they can protect the human body against deterioration by free radicals and help to prevent the diseases. The use of synthetic antioxidants must be controlled because of potential health hazards. So, the search for natural antioxidants as safe alternatives to synthetic products is important in the food industry. Thus, the use of natural antioxidants present in food and other biological substances has attracted significant interest due to their safety and nutritional values and also therapeutic values. Siddha system mainly based on natural resources which having antioxidant, immunomodulator effects. These properties reduce and prevent the disease prevalence. Kadukkai (*Terminalia chebula)* is the most popular herb which contains

phytoconstituents and also which was scientifically proved potent antioxidant property which helps to prevent the seasonal diseases and also NCDs.

REFERENCES

1. Anamika Gautam, Shashi Jain., "Effect of Lifestyle on Prevention of Non-Communicable Diseases", International Journal of Science and Research (IJSR), Volume 5 Issue 12, December 2016.
2. Evans, Mark D et al. "Oxidative DNA damage and disease: induction, repair and significance." *Mutation research* vol. 567, 1 (2004): 1-61.
3. https://www.nhp.gov.in/healthlyliving/ncd (2019) National health portal of India.
4. Seyedsadjadi, N., & Grant, R, "The Potential Benefit of Monitoring Oxidative Stress and Inflammation in the Prevention of Non-Communicable Diseases (NCDs)" *Antioxidants (Basel, Switzerland)*, 2020, *10*(1), 15.
5. Kuo PC, Damu AG, Cherng CY, et al., "Isolation of a natural antioxidant, dehydrozingerone from *Zingiber officinale* and synthesis of its analogues for recognition of effective antioxidant and antityrosinase agents", Arch Pharm Res, 28:518–28.
6. Lobo V, Patil A, Phatak A, Chandra N, "Free radicals, antioxidants and functional foods:Impact on human health" *Pharmacogn Rev*. 2010;4(8):118-126.
7. Thiyagarajan R, "*Siddha MaruthuvamSirappu*" 1st ed. Directorate of Indian Medicine and Homeopathy; Chennai-106; 2013.
8. R.R. Chattopadhyay et al, Phcog Rev, "Plant Review *Terminalia chebula*: An update" Pharmacognosy Reviews, Vol 1, Issue 1, Jan- May, 2007.
9. Anwesa Bag, Subir Kumar Bhattacharyya, Rabi RanjanChattopadhyay, "The development of Terminalia chebula Retz. (Combretaceae) inclinical research" Asian Pac J Trop Biomed, 2013; 3(3): 244-252.
10. "Indian herbal pharmacopeia" Indian drug manufacturers' association, Mumbai. Revised new edition 2002.pg.no:439-446.
11. MurugesamuthaliyarK.C, Siddha Materia Medica (Medicinal Plants Division). 2nd ed. Directorate of Indian Medicine and Homeopathy, Chennai-106.2008.
12. Indhu PM, SherinNisha A et al., "*Kadukkai ooralkudineer*(Chebulic Soaked Water): Prophylactic Siddha Herbal Drink for Covid-19" Journal of Siddha, 4(1), 37-47.
13. MariappanAmutha et al., "A Pilot Study on The Determination of Antioxidant Potential and Lethal Dosage of Hydro Alcoholic Fruit Extract of *Terminalia Chebula"* International Journal Of Pharmacy And Pharmaceutical Sciences, Vol 6, Issue 6, 2014.
14. Neelam Kushwaha et al., "Phytochemical analysis and assessment of *in vitro* antioxidant properties of different plants", Journal of Pharmacognosy and Phytochemistry 2017; 6(3): 123-130.
15. Chia Lin Chang and Che San Lin, "Phytochemical Composition, Antioxidant Activity, and Neuroprotective Effect of *Terminalia chebula*Retzius Extracts", Evidence-Based Complementary and Alternative Medicine,2012.
16. Kumar KJ, "Effect of geographical variation on contents of tannic acid, gallic acid, chebulinic acid and ethyl gallate in *Terminalia chebula*", Natural Products, 2006;2(3- 4):170-75.
17. Juang LJ, Sheu SJ, Lin TC,"Determination of hydrolyzable tannins in the fruit of *Terminalia chebula*Retz. by high-performance liquid chromatography and capillary electrophoresis", J Sep Sci 2004;27(9):718–24.
18. William C Evans,Trease and Evan's, "Pharmacognosy", 16th Ed., W.B. Saunders Co. Pvt.Ltd., 1996, page no:232.
19. G.P. Senthilkumar& S. Subramanian, "Evaluation of Antioxidant Potential of *Terminaliachebula*. Fruits Studied in Streptozotocin-Induced Diabetic Rats", Pharmaceutical Biology, (2007), 45:6, 511-518.
20. Hazra B, Sarkar R, Biswas S, Mandal N, "Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis*", BMC Complement Altern Med. 2010 May 13;10:20.
21. Saha S, Verma RJ, "Antioxidant activity of polyphenolic extract of *Terminalia chebula* Retzius fruits", J Taibah Univ Sci, 2016; 10(6):805-812.

22. Chen X., Sun F., Ma L., Wang J., Qin H., Du G, "*In vitro* evaluation on the antioxidant capacity of tri-ethylchebulate, an aglycone from *Terminalia chebula* Retz fruit", Indian Journal of Pharmacol2011; 43(3):6.
23. Saleem A, Ahotupa M, Pihlaja K, "Total phenolics concentration and antioxidant potential of extracts of medicinal plants of Pakistan", Z Naturforsch C J Biosci. 2001 Nov-Dec;56(11-12):973-8.
24. Hyun-Sun LEE et al., "Antioxidant Effects of Aqueous Extract of *Terminalia chebula in Vivo* and *in Vitro*", *Biol. Pharm. Bull.* 28(9) 1639—1644 (2005).
25. Klika, Karel & Saleem, Ammar &Sinkkonen, Jari&Kähkönen, Marja&Loponen, Jyrki &Tähtinen, Petri &Pihlaja, Kalevi, "The structural and conformational analyses and antioxidant activities of chebulinic acid and its thrice-hydrolyzed derivative, 2,4-chebuloyl-β-D-glucopyranoside, isolated from the fruit of *Terminalia chebula*", ARKIVOC: archive for organic chemistry. 7. 83-105.
26. Vemuri P. K, Dronavalli L, Nayakudugari P, Kunta A, Challagulla R, "Phytochemical Analysis and Biochemical Characterization of *Terminalia Chebula* Extracts For Its Medicinal Use" ,Biomed Pharmacol J 2019;12(3).
27. Harpreet Walia, Subodh Kumar and Saroj Arora, "Comparative analysis of antioxidant and phenolic content of chloroform extract/fraction of *Terminalia chebula*", Journal of Basic and Clinical Pharmacy,Vol-002 Issue-003 August 2011.
28. Mahesh R, Bhuvana S, Begum VM, "Effect of *Terminalia chebula* aqueous extract on oxidative stress and antioxidant status in the liver and kidney of young and aged rats" Cell BiochemFunct. 2009 Aug; 27(6):358-63.
29. Lee HS, Jung SH, Yun BS, Lee KW, "Isolation of chebulic acid from *Terminalia chebula* Retz. and its antioxidant effect in isolated rat hepatocytes", Arch Toxicol. 2007 Mar; 81(3):211-8.
30. Chen X, Sun F, Ma L, Wang J, Qin H, Du G, "In vitro evaluation on the antioxidant capacity of triethylchebulate, an aglycone from *Terminalia chebula* Retz fruit", Indian J Pharmacol. 2011 May; 43(3):320-3.
31. Rattanasena P, "Antioxidant and antibacterial activities of vegetables and fruits commonly consumed in Thailand" Pak J Biol Sci. 2012 Sep 15;15(18):877-82.
32. Sheng Z, Zhao J, Muhammad I, Zhang Y, "Optimization of total phenolic content from *Terminalia chebula* Retz. fruits using response surface methodology and evaluation of their antioxidant activities", Plos one. 2018; 13(8).
33. T. Vani, M. Rajani, S. Sarkar & C. J. Shishoo, "Antioxidant properties of the Ayurvedic formulation*Triphala* and its constituents", International Journal of Pharmacognosy, 35:5, 313-317.
34. Cheng HY, Lin TC, Yu KH, Yang CM, Lin CC. "Antioxidant and free radical scavenging activities of *Terminalia chebula*" Biol Pharm Bull. 2003 Sep; 26(9):1331-5.
35. MariappanAmutha et al., "A Pilot Study On The Determination Of Antioxidant Potential And Lethal Dosage Of Hydro Alcoholic Fruit Extract of *Terminalia Chebula*", International Journal Of Pharmacy And Pharmaceutical Sciences,Vol 6, Issue 6, 2014.
36. Naik GH, Priyadarsini KI, Naik DB, Gangabhagirathi R, Mohan H, "Studies on the aqueous extract of *Terminalia chebula* as a potent antioxidant and a probable radioprotector", Phytomedicine. 2004 Sep;11(6):530-8.
37. Suchalatha, Subramaniyan&Srinivasulu, Chennam& Devi, Shyamala, "Antioxidant activity of ethanolic extract of Terminalia chebula fruit against isoproterenol-induced oxidative stress in rats", Indian journal of biochemistry & biophysics. 42. 246-9.

Table.No:1. *Kadukkai karpam* and its *anupanam* for various seasons

***Kalangal* (seasons)**	***Anupanam* (vehicle)**
Kaarkalam (Monsoon)	*Induppu* (Rock salt)
Koothirkalam (Autumn)	*Sarkarai* (Sugar)
Munpani(Early winter)	*Chukku* (Dried ginger)
Pinpani (Late winter)	*Thippili* (Long pepper)
Elavenil (Spring)	*Thean* (Honey)
Muthuvenil (Summer)	*Vellam* (Jaggery)

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RESEARCH ARTICLE

Impact of GST on Investors Decision with Special Reference to Automobile Industry

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ABSTRACT

This study "Impact of GST on Investors Decision with special reference to Automobile Industry" clearly demonstrated the role of GST in automobile industry. In our country the automobile industries is also one among the top most revenue generating and directly promote the economic growth. Research design used in this study was descriptive research helps to determine the perception of GST among investors and consumers of automobile industry. The statistics in the current study showcased that majority of the employees that there was a substantial increase in their sales after the arrival of the GST regime. The major aim of the GST implementation is to reduce the multiple taxation system. Based on the findings suggest that there should be audits every year like under direct taxes rather than for a block period of 5 years.

Keywords: Goods and Services Tax (GST), Economic Development, Automobile Sector, Gross Domestic Product (GDP), Globalization.

INTRODUCTION

GST is an indirect tax levied by the Central Government, under the Constitutions Amendments. It is a Uniform tax system; there is a more benefit to the business firms and retail sector. In India, the GST is designed to give a world class tax system and to improve the tax collections. In Our Country, the automobile sector is the key player of highly contributing to the nation's Gross Domestic Product (GDP). The main aim of the Indian Automobile Industry is to acquire top position in manufacturing and exporting fields. It also aims to increase the contribution of Gross Domestic Product rate. The imposition of GST helps the automobile sector by reducing the manufacturing tax rates.

While focusing before GST tax rate in automobile sector the industry was required to pay the multiple tax system. The impact of GST could also be used to the consumer, dealer and manufacturers. So the consumers who are all the end user of the automobile receive the benefits of tax system on purchase of vehicles. After implementing the GST the automobile sector has to pay unified tax system under the CGST and SGST.

Review of Literature

Rowchoudhary (2012), the indirect taxation structure led towards the lowering of productivity as means of decrease in economic growth along with other development and international trade of Indian market. The indirect taxation system required immediate reformation in the taxation structure concerning the taxation on goods and services in order to maintain the economy of India. The existing taxation system was replaced by the new taxation structure. The new taxation system requirement was needed to recover the crisis caused to the economy of India and improve the economy to a greater extent. According to Garg (2014), defined It has been also estimated by one of the researchers that GST introduction in taxation structure of India is the logical movement towards indirect tax reformation in India. Dani (2016), the GST regime has also been considered as a half-hearted attempt in the indirect taxation system of India the GST is not only implemented in India but 150 more countries have followed the concept GST implementation. The researcher suggested that the Indian government should study the GST regime of the different countries along with drawbacks bore its implementation in India. The fact is clear that GST implementation has simplified indirect taxation in India and has proved to be efficient in removal of heterogeneous taxation structure involving the issues of threshold limit, petroleum products, real estate, revenue rate, etc.

Objectives of the Study

- To analyze the impact of GST on the automobile sector.
- To study the benefits and challenges that GST has on the automobile sector.

RESEARCH METHODOLOGY

Research Design: Descriptive Research Design is used in the study to determine and analyse the role of GST in automobile sector. This design helps the researcher to understand the various factors and its relationship in the automobile sector.

Sampling technique: Non probability sampling techniques – Judgemental Method was adopted in this research. Sample size 300 respondents. Primary data collected by using structured questionnaire. Secondary data collected from journals, books and official gazette. Data Analysis by using the descriptive statistics, Chi square test and Kruskal Walli Test. H0: There is no benefits and challenges that GST has on the automobile sector.

In the table 1 p-value is less than 0.05 in the sig column indicating that it is significant so we reject H0 and conclude that there are benefits and challenges that GST has on the automobile sector. H0: There is no future implication on the automobile sector. In the table 2 p-value is less than 0.05 in the sig row indicating that it is significant so we reject H0 and conclude that there are future implications on the automobile sector. H0: There is no growth driver across the automobile sector with respect to GST. In the table 3 p-value is less than 0.05 in the sig column indicating that it is significant so we reject H0 and conclude that there is impact of GST on the automobile sector.

Findings

Further, it was discovered through the survey that employees also affirmed that GST has an impact on the valuation. Valuation impact refers to the notion that the taxable supply of the goods is provided by the person that deals with the selling and buying of second-hand goods. As a result of this, it creates a difference in the supply value between the selling price and the value of purchasing. This directly affects the automobile sector because the GST is only paid on the difference value. Moreover, it was illustrated that there existed complexities in the bifurcation of the material

and labour components in concern to the servicing of vehicles. This creates a cause of conflict which results in disputes at multiple scaffolds. This was mainly because the authorities that are responsible for both the service tax and sales tax demand taxes on a higher component. These issues affect the operations of the automobile industry detrimentally.

Among the varied benefits and implications of GST, it was unveiled that many employees strategically delineated that the GST is a fair and just tax system. Furthermore, it was also outlined that the registration procedure under the GST regime was deemed to be simple. The statistics in the current study showcased that majority of the employees that there was a substantial increase in their sales after the arrival of the GST regime. Moreover, it was also found that employees in the current study agreed with the notion that the GST has brought transparency in business transactions. Findings of the current study wherein, the majority of the employees affirmed the notion that they faced technical system issues of the GSTN portal. Furthermore, it was outlined that lack of trained manpower in the company is another issue faced by the employees in the automobile sector in the implementation of GST.

Suggestions

Based on the findings acquired it can be elucidated that there is no adverse impact of GST on the automobile sector. Varied queries were made with the employees in concern to the factors or practice they considered as drivers across the automobile sector concerning GST in this regard it was delineated that the majority of the employees to suit the needs of GST of breaking up into the multiple supplies, composite supplies or missing the supplies was conductive to the growth of the automobile sector. Secondly, all state level taxes should be agglomerated into the SGST. The major aim of the GST implementation is to reduce the multiple taxation system. Findings further suggest that there should be audits every year like under direct taxes rather than for a block period of 5 years.

CONCLUSION

Our Indian Economy is growing more and more globalized, therefore a need of uniform and standard tax rate system is to promote all the sectors. Goods and Services Tax based on liberal in assessment and ruthless in collection. GST focus on greater transparency, neutrality in tax rate facilitates MSME and minimum compliance requirements. This GST helps to increase the growth of GDP and promote the economic development.

REFERENCES

1. Chaurasia, P., Singh, S., & Sen, P. K (2017), Role of Good and Service Tax in the Growth of Indian economy. International Journal of Science, Technology and Management, 5 (2), 152-157.
2. Dash A (2017), Positive and negative impact of GST on Indian economy. *International Journal of Management and Applied Science*, *3*(5), 158-160.
3. Dash, Bharatee Bhusana; Raja, Angara V (2013),"Intergovernmental Transfers and Tax Collection in India: Does the Composition of Transfers Matter?" In "Public Budgeting & Finance", Blackwell Publishing Ltd(Maiden
4. Poddar, S & Ahmad, E (2009), GST reforms and intergovernmental considerations in India. Ministry of Finance, Government of India.
5. Subodh Kumawat (2018), GST Impact on Automobile and Spare Parts Industry in India https://blog.saginfotech.com/gst-impact-on-automobile-industry-in-india
6. MN, A. (2016), Impact of Goods and Service Tax (GST) on Logistics Sector in India. *Chronicle*.
7. Sharma, A., & Sharma, D. Impact of GST on Automobile Industry in India.
8. Pandey, K. K. (2020), a New Tax Regime: GST Consolidation of Multiple Taxes and its Impact on Automobile Sector. *Studies in Indian Place Names*, *40*(56), 1518-1524.
9. Singh, D (2018), Automobile sector Effected by GST.

Table 1

Coefficients[a]								
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	1.157	.788		1.468	.143		
	E1	.131	.106	.131	1.245	.215	.353	2.832
	E2	-.081	.123	-.066	-.663	.508	.398	2.514
	E3	-.018	.154	-.014	-.119	.905	.276	3.629
	E4	.056	.155	.047	.360	.720	.223	4.478
	E5	.303	.196	.141	1.548	.123	.469	2.130
	E6	.084	.061	.101	1.385	.167	.724	1.382
	E7	.003	.126	.003	.028	.978	.303	3.296
	E8	.187	.138	.130	1.355	.177	.423	2.367
	E9	-.038	.130	-.036	-.290	.772	.254	3.935
	E10	-.124	.205	-.100	-.606	.545	.144	6.957
	E11	-.210	.144	-.141	-1.465	.144	.422	2.370
	E12	-.060	.062	-.073	-.972	.332	.681	1.469
	E13	.203	.115	.184	1.761	.080	.357	2.799
	E14	-.345	.164	-.263	-2.099	.037	.247	4.055
	E15	.200	.179	.126	1.117	.265	.304	3.286
a. Dependent Variable: age								

Table 2

Test Statistics[a,b]										
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Chi-Square	5.878	12.584	10.790	12.644	9.936	14.937	4.253	5.053	12.419	20.455
Df	4	4	4	4	4	4	4	4	4	4
Asymp. Sig.	.208	.013	.029	.013	.042	.005	.373	.282	.014	.000
a. Kruskal Wallis Test										
b. Grouping Variable: education										

Table 3

Coefficients[a]								
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	3.212	.571		5.625	.000		
	D1	.143	.094	.121	1.527	.128	.588	1.700
	D2	-.212	.087	-.195	-2.441	.015	.584	1.712
	D3	-.142	.071	-.140	-1.993	.047	.755	1.324
	D4	-.047	.085	-.043	-.553	.581	.603	1.659
	D5	.108	.071	.120	1.530	.127	.599	1.668
	D6	.110	.084	.107	1.313	.190	.558	1.792
	D7	-.093	.095	-.088	-.987	.324	.472	2.117
	D8	.044	.099	.040	.443	.658	.454	2.202

	D9	-.120	.073	-.117	-1.630	.104	.719	1.391
	D10	.015	.084	.012	.177	.860	.833	1.200
	D11	.359	.086	.376	4.181	.000	.460	2.174
	D12	-.308	.105	-.245	-2.936	.004	.534	1.871
a. Dependent Variable: age								

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RESEARCH ARTICLE

In silco Analysis of Versatile Plant based Compounds from *Thuja occidentalis* in Modulating ion Channels Responsible for Triple Negative Breast Cancer

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ABSTRACT

The modulation of different ion channels has been reported in the progression of triple negative breast cancer. Triple-negative breast cancer is a type of breast cancer that doesn't express estrogen and progesterone receptors and doesn't have HER-2 genes. So it very difficult to treat with available hormone therapy. But it is very interesting to note the role of certain ion channels in triple negative breast cancer. In this work we used compounds from *Thuja occidentalis* and identify its action in modulating the aquaporin channel 5 and chloride channel 3 which is responsible for the progression of triple negative breast cancer through *in silco* analysis. Through this study we identified that p-coumaric acid, Umbelliferone, Fenchone and Thujone is effective in modulating AQP5 and chloride channel 3 in triple negative breast cancer.

Keywords: Triple-negative breast cancer, aquaporin channel 5, chloride channel 3, Thuja occidentalis, *In silco* analysis.

INTRODUCTION

Breast cancer is a common malignancy found in women with different age groups. But the seriousness is that the number of patients with this malignancy is increasing each year[1]. So it has become a common threat to the life of many women's. Breast cancer is a heterogeneous group of diseases. It is divided into different subtypes based on three receptors. They are estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) [2]. Triple negative breast cancer or TNBC is the one which doesn't express estrogen receptor

(ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2)[3]. Since it doesn't express these receptors, it doesn't responds to the hormonal therapy and other available targeted agents. Moreover it has higher rate of metastasis and can relapse after treatment [4]. The ion channel plays a significant role in mammary gland physiology. It has an important role in initiation as well as progression of breast cancer [5].AQP channels or aquaporin channels are the family of transmembrane protein which play a significant role in transportation of water and small solutes in and out of the cell [6]. There are about 13 different aquaporin's in which the expression of AQP1, AQP3-5 and AQP10-12 is identified in normal human mammary gland, which helps in milk secretion [7]. In these an aquaporin channel called AQP5 has a significant oncogenic property by prompting angiogenesis, metastasis and enhancing the transport of ROS [8]. The expression of the AQP5 is upregulated in Triple negative breast cancer [9]. The overexpression of AQP5 in Triple negative breast cancer is seen in the membrane and cytoplasm of cancer cell which in turn result in the poor prognosis of cancer [10].

Likewise chloride channel 3 also plays a significant role in the progression of triple negative breast cancer. Chloride channels are ion channels which play an indispensable role in the transportation of Cl^- [11]. There are about 9 different types of Chloride channels which are divided into 2 main groups based on their distribution and its physiological function. The chloride channel 3 is basically found in vascular intima of the endosome-lysosomal pathway [12]. The extent of chloride channel 3 in plasma membrane is low due to protein degradation and hydrolase activity [13]. The over expression of chloride channel 3 had been observed in triple negative breast cancer [14]. The knockdown of chloride channel 3 down regulates the expression of cyclins and increases levels of p21 which indicates that knockdown of this channels can block the cell cycle of triple negative breast cancer cells at G0/G1 phase which inhibits the cell proliferation [15].

The chloride channel 3 and AQP5 is a promising new targets for the treatment of triple negative breast cancer. Plants are good source of medication for a long period of time. The plants based compounds specifically targeting chloride channel 3 and AQP5 will be good therapeutic option against triple negative breast cancer. *Thuja occidentalis* which is also known as white cedar belongs to the *Cupressaceae* family [16]. This plant has been used in traditional medicine for the treatment of various carcinoma as well as many another diseases including liver diseases, bullous bronchitis, amenorrhea, cystitis, psoriasis, rheumatism etc. The recent studies shows that it has antioxidant[17], anti-inflammatory[18], anti-microbial [19] and anticancer property [20]. The biological activity is because it contain a large number of chemical compounds including essential oil, Coumarins, flavonoids, tannins and proanthocyanidines [21].In this work, we are suggesting some of *Thuja occidentals* compounds as a lead molecule in modulating chloride channel 3 and AQP5 through computational bioinformatics analysis.

MATERIALS AND METHODS

Bioactive compound selection

Compounds of *Thuja occidentalis* plants were studied from several literature. Chemical structures and analogs of the extracted compounds from *Thuja occidentalis*were searched in chemical databases like PubChem (http://pubchem.ncbi.nlm.nih.gov/).

Pharmacokinetic profiling

The compounds from *Thuja occidentals* is undergone Insilco based validation studies to understand their pharmacokinetic activities. In this study, ADMET profile (Absorption, distribution, metabolism, elimination and toxicity)of the identified compound is analysed by Swiss ADME as an analytical tool. All compounds were analyzed based on their default threshold values such as blood brain barrier (BBB), solubility, Cytochrome P450 oxidase enzyme (CYP2D6) activity and pgp substrate.

Target protein selection and active site prediction

The target proteins such asAQP5 (PDB ID:3D9S) and Chloride channel 3(PDB ID:3KJY) are retrieved from PDB website. Active site of the two protein is identified by using PyMOL and CASTp analysis.

Molecular interaction studies of drug like compounds from *Thuja occidentalis*

Molecular interaction studies were done in order to understand the strength of interactions of selected compounds. For that Affinity, intermolecular binding energy, Vander walls energy and electrostatic energy is identified by using DockThor.

RESULTS AND DISCUSSION

Bioactive compounds from *Thuja occidentalis* plant and its analogs.

From the literature survey we have identified different compounds isolated from *Thuja occidentalis*. We also identified analogs of the same compounds by similarity based search. The compounds are classified under essential oil, coumarins, flavonoids, tannins and proanthocyanidines. The different essential oil are Thujone, borneol, camphene, fenchone, limonene, myrcene and the two coumarins which found in *Thuja occidentalis* are p-coumaric acid and umbelliferone. Kaempferol, myricitrin and quercetin are the different flavonoids which is found in*Thuja occidentalis*. Catechine which is a tannin and Procyanidin and Prodelphinidin are the two Proanthocyanidines found in *Thuja occidentalis*. Hence all the compounds from the plants and the number of retrieved analogs obtained from chemical databases are tabulated in Table 1.

Pharmacokinetics studies of compoundsfrom *Thuja occidentalis*

The compounds from *Thuja occidentalis*is screened for pharmacokinetic study. Unwanted compounds were removed based on the violations in ADMET(Absorption, distribution, metabolism, elimination and toxicity) descriptors. As a result of this screening, certain compounds were eliminated due to the failure in the confidence levels such as low solubility, levels of penetration in blood brain barrier (BBB) as well as high toxicity in ADMET descriptor analysis. From the result of screening, we have found versatile compounds in *Thuja occidentalis* having a good bioavailability score. We omit certain compounds which is poor in either adsorption or BBB. We also omit certain compounds which is a Pgp substrate or CYP2D6 inhibitor. As a result of this study, we found only certain compounds which passed all levels of ADMET screening and results are given in the Table 2. Compounds with strong pharmacokinetic strength were taken for study. The selected compounds includeBorneol, Fenchone, p-coumaric acid, Umbelliferone and Thujone. The selected compounds were allowed for molecular docking analysis.

Protein and Binding sites

Active site of the two proteins was identified by using PyMOL and CASTp analysis. From the active site search, of AQP5 channelwe found that LEU 47, THR 155, THR 156, PRO 157, VAL 158, GLY 159, SER 160, LEU 163 AND LEU 167 is responsible for the inhibitory mechanism. Likewise from the active site search, of chloride channel 3 we found that ARG 27, HIS 110, LYS 111, SER 222, HIS 148 and ASP 168 is responsible for the inhibitory mechanism. The active site residues of the secondary structure of proteinAQP5 and channel 3were given in Fig. 1

Molecular docking analysis

Molecular docking was performed by using DockThor. Using this program, the two proteins AQP5 and chloride channel 3were docked against the selected compounds such asBorneol, Fenchone, p-coumaric acid, Umbelliferone and Thujone. Each compound have different kind of interaction with the two channel protein, which provide with the maximum docking score. The docking results was then analyzed to identifying the compounds having maximum affinity and minimum total binding energy for docking. From that we identified that four compounds having

maximum affinity and have minimum total energy for docking. The compounds are p-coumaric acid, Umbelliferone, Fenchone and Thujone. These compounds show maximum affinity and have minimum total energy with AQP5 and chloride channel 3.the results of the docking is given in the table 3 and 4.

CONCLUSION

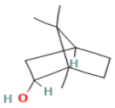
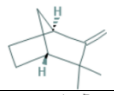
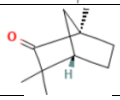
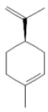
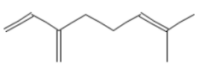
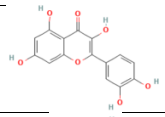
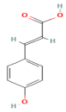
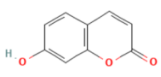
The use of compounds from plant source with pharmacological importance is a therapeutic approach of modern medication against different type of cancers. According to various studies, the use of plant compounds are reported to bethe most reliable and efficient with therapeutic significance. In this Insilco study the versatile plant based compounds from *Thuja occidentalis* having action in modulating the two different ion channels i.e. AQP5 and chloride channel 3 responsible for triple negative breast cancer is studied. This provide an insight for developing novel compounds against this cancer. The inhibitory efficiency of the compounds were analyzed through the docking studies using target protein and the ligand molecules provide an insight of using compounds against triple negative breast cancer. Through this study we identified that p-coumaricacid, Umbelliferone, Fenchone and Thujone is effective in modulating AQP5 and chloride channel 3 in triple negative breast cancer.

REFERENCES

1. DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL. Breast cancer statistics, 2019. CA Cancer J Clin. 2019;69(6):438–51.
2. Jafari SH, Saadatpour Z, Salmaninejad A, Momeni F, Mokhtari M, Nahand JS, Rahmati M, Mirzaei H, Kianmehr M. Breast cancer diagnosis: imaging techniques and biochemical markers. J Cell Physiol. 2018;233(7):5200–13.
3. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, Shi B. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res. 2015;5(10):2929–43.
4. Jhan JR, Andrechek ER. Triple-negative breast cancer and the potential for targeted therapy. Pharmacogenomics. 2017;18(17):1595–609.
5. Ma Z, Yuan D, Cheng X, Tuo B, Liu X, Li T. Function of ion transporters in maintaining acid-base homeostasis of the mammary gland and the pathophysiological role in breast cancer. Am J PhysiolRegulIntegr Comp Physiol. 2020;318(1):R98-r111.
6. Wang L, Zhang Y, Wu X, Yu G. Aquaporins: new targets for cancer therapy. Technol Cancer Res Treat. 2016;15(6):821–8.
7. Mobasheri A, Barrett-Jolley R. Aquaporin water channels in the mammary gland: from physiology to pathophysiology and neoplasia. J Mammary Gland Biol Neoplasia. 2014;19(1):91–102.
8. Kasa P, Farran B, Prasad GLV, Nagaraju GP. Aquaporins in female specific cancers. Gene. 2019;700:60–4
9. Dajani S, Saripalli A, Sharma-Walia N. Water transport proteins-aquaporins (AQPs) in cancer biology. Oncotarget. 2018;9(91):36392–405.
10. Zhu Z, Jiao L, Li T, Wang H, Wei W, Qian H. Expression of AQP3 and AQP5 as a prognostic marker in triple-negative breast cancer. Oncol Lett. 2018;16(2):2661–7.
11. Abeyrathne PD, Chami M, Stahlberg H. Biochemical and biophysical approaches to study the structure and function of the chloride channel (ClC) family of proteins. Biochimie. 2016;128–129:154–62.
12. Jentsch TJ. Discovery of CLC transport proteins: cloning, structure, function and pathophysiology. J Physiol. 2015;593(18):4091–109.
13. Stölting G, Fischer M, Fahlke C. CLC channel function and dysfunction in health and disease. Front Physiol. 2014;5:378.
14. Yang H, Ma L, Wang Y, Zuo W, Li B, Yang Y, Chen Y, Chen L, Wang L, Zhu L. Activation of ClC-3 chloride channel by 17β-estradiol relies on the estrogen receptor α expression in breast cancer. J Cell Physiol. 2018;233(2):1071–81.

15. Zhou FM, Huang YY, Tian T, Li XY, Tang YB. Knockdown of Chloride Channel-3 Inhibits Breast Cancer Growth In Vitro and In Vivo. J Breast Cancer. 2018;21(2):103–11.
16. Silva, I.S.; Nicolau, L.A.D.; Sousa, F.B.M.; de Araújo, S.; Oliveira, A.P.; Araújo, T.S.L.; Souza, L.K.M.;Martins, C.S.; Aquino, P.E.A.; Carvalho, L.L.; et al. Evaluation of anti-inflammatory potential of aqueousextract and polysaccharide fraction of Thuja occidentalis Linn. in mice. Int. J. Biol. Macromol. 2017, 105,1105–1116.
17. Bellili, S.; Aouadhi, C.; Dhifi, W.; Ghazghazi, H.; Jlassi, C.; Sadaka, C.; El Beyrouthy, M.; Maaroufi, A.;Cherif, A.; Mnif, W. The influence of organs on biochemical properties of tunisian Thuja occidentalis essentialoils. Symmetry 2018, 10, 649.
18. Caruntu, S.; Olah, N.K.; Balta, C.; Rosu, M.; Mladin, B.; Hermenean, A. Thuja occidentalis mother tincture alleviate the COX-2 expression in intestinal mucosa of the TNBS-induced ulcerative colitis model in mice. Studia Univ. VasileGoldi¸s Ser. ¸Stiin¸teleVie¸tii 2020, 30, 145–148.
19. Asha, R.; Nisha, P.; Suneer, K.; Mythili, A.; Shafeeq Hassan, A.; PanneerSelvam, K.; Manikandan, P.; Shobana, C.S. In vitro activity of various potencies of homeopathic drug Thuja against molds involved in mycotic keratitis. Internat. J. Pharm. Pharm. Sci. 2014, 6, 555–559.
20. Sunila, E.S.; Kuttan, G. A preliminary study on antimetastatic activity of Thuja occidentalis L. in mice model. Immunopharmacol. Immunotoxicol. 2006, 28, 269–280
21. Wei Tian, Chang Chen, Xue Lei, Jieling Zhao, Jie Liang, CASTp 3.0: computed atlas of surface topography of proteins, Nucleic Acids Research, Volume 46, Issue W1, 2 July 2018, Pages W363–W367

Table 1. Bioactive plants and their derivatives and number of retrieved analog compounds

Compounds	Structure	No of Analogs
Borneol		285
Camphene		228
Fenchone		454
Limonene		2991
Myricene		339001
Quercetin		5202
p-coumaric acid		670
Umbelliferone		1487

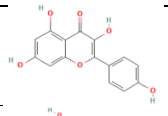
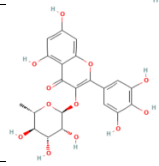
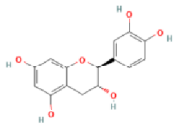
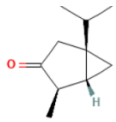
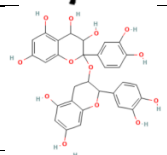
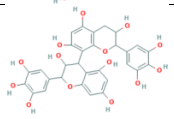
Kaempferol		4996
Myricitrin		11704
Catechine		3004
Thujone		159
Procyanidin		1496
Prodelphinidin		1578

Table 2. ADMET screening of different compounds of *Thuja occidentalis*

Compounds	Water Solubility	GI absorption	BBB permeant	Pgp substrate	CYP2D6 inhibitor	Bioavailability Score
Borneol	Soluble	High	Yes	No	No	0.55
Camphene	Soluble	Low	Yes	No	No	0.55
Fenchone	Soluble	High	Yes	No	No	0.55
Limonene	Moderately soluble	Low	Yes	No	No	0.55
Myricene	Moderately soluble	Low	No	No	No	0.17
Quercetin	Soluble	High	No	No	Yes	0.55
p-coumaric acid	Soluble	High	Yes	No	No	0.85
Umbelliferone	Soluble	High	Yes	No	No	0.55
Kaempferol	Soluble	High	No	No	Yes	0.55
Myricitrin	Moderately soluble	Low	No	No	No	0.17
Catechine	Moderately soluble	Low	No	No	No	0.17
Thujone	Soluble	High	Yes	No	No	0.55
Procyanidin	Moderately soluble	Low	No	No	No	0.17
Prodelphinidin	Moderately soluble	Low	No	No	No	0.17

Table 3 Docking score of AQP5 against selected compounds

Compounds	Affinity	Total energy	Vdw Energy	Electrical energy
Borneol	-7.847	3.160	-14.001	-2.760
Fenchone	-6.99	-0.852	-11.18	-3.927
p-coumaric acid	-6.48	-10.924	-12.29	-12.187
Umbelliferone	-6.88	-2.733	-11.01	-10.151
Thujone	-6.90	-1.22	-12.9	-12.22

Table 4 Docking score of AQP5 against selected compounds

Compounds	Affinity	Total energy	Vdw Energy	Electrical energy
Borneol	-8.001	0.456	-2.247	-10.991
Fenchone	-6.997	-4.043	-6.84	-11.684
p-coumaric acid	-5.833	-16.936	-9.150	-21.722
Umbelliferone	-6.661	-9.640	-5.760	-22.522
Thujone	-7.011	-1.043	-3.84	-11.184

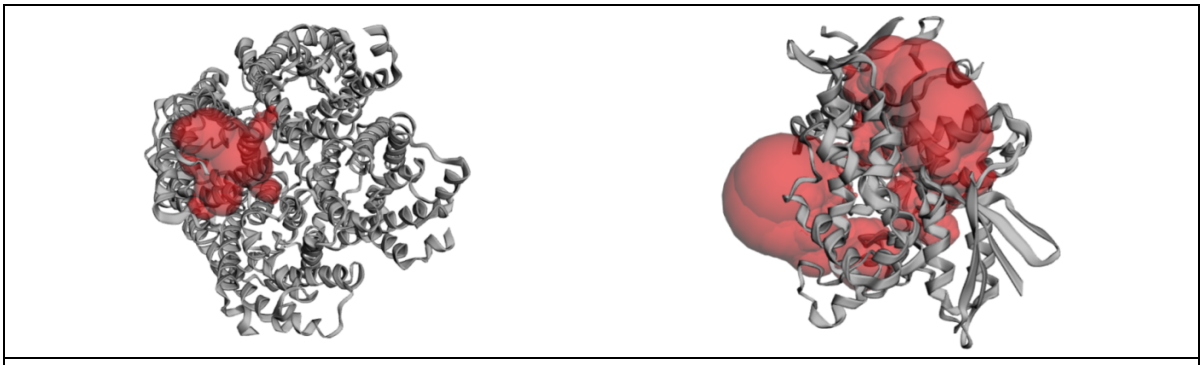


Fig. 1. Predicted active site pocket in entire structure ofAQP5 and chloride channel 3

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RESEARCH ARTICLE

Stewardable Organizational Citizenship Behavior: A Two-Wave Study

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ABSTRACT

The primary purpose of this article is to analyze the influence of stewardship behavior dimensions on organizational citizenship behavior. This paper also examines the mediating effects of idiosyncratic deals on stewardship behavior towards organizational citizenship behavior. This paper attempts to partially fill the research gap by using stewardship behavior as the antecedents of organizational citizenship behavior, which is mediated by idiosyncratic deals. Data were accumulated from 226 employees of spinning millings registered with South India Spinners Association using two-wave cross-lagged self-reported questionnaires. The proposed hypotheses are tested using correlation, path analysis, and structural equation modeling. The results revealed that there are significant positive correlations between organizational identification, collectivist orientation, and use of personal power and organizational citizenship behavior. Future studies could focus on the impact of non-negotiated contracts (psychological contract) on other in-role and extra-role behaviors of employees.

Keywords: Stewardship Behavior, Organizational Citizenship Behavior, Idiosyncratic Deals

INTRODUCTION

Considering the dynamic business environment, organizations hinge upon on employees' extra-role behaviors to bushel the gaps between role behaviors identified on job descriptions and those created by contemporary demand. An individual's behavior that profit the organization but for which the employee is not formally rewarded by the organization is referred as organizational citizenship behavior (Nurjannah, 2019). The implication of organizational citizenship behavior in increasing organizational potency in an industrious context is apparent in several

examinations. The stewardship theory states that the usefulness and gratification that effuse from achieving success that originate from tremendous utilization of resources is ampler than focusing on short-term benefits. In stewardship behavior, individuals represses personal interests and short-term benefits for larger good and selfless accomplishments as a trustable employee of organizational resources (Kuppelwieser, 2011). Furthermore, in an operating context, that progressively encourages the flexibility and laissez faire of the employment relationship, idiosyncratic deals are growing as a common practice. Idiosyncratic deals can be referred as a form of customization allowing workers particular circumstances varying from their counterparts doing the same job (Anand and Vidyarthi, 2015).

Problem Statement

Organizational citizenship behavior being fragmented from the conventional reward system, it is essential to investigate the key antecedents of organizational citizenship behavior among the workforce so that management can actuate employees to acquit such advantageous behaviors. The multifariousness of atmospheric condition in the organization may create individuals to perceive idiosyncratic deals negatively by making social comparisons between team members as few employees experience prosperous treatment whereas others do not. On the one hand, employees who are disadvantaged of idiosyncratic deals may think that they are handled unfairly corresponded with the idiosyncratic deals recipient. On the other hand, the idiosyncratic deals receiver gains from resources that put them at a favorable position compared to their workfellows. Nevertheless, it is still not clear about how idiosyncratic deals affect the outcomes in terms of organizational citizenship behavior.

Purpose of the Study

A steward's behaviour of an individual is organisation-centred rather than individual-centred. In addition, a steward acquires gratification from the success of the organization. Accordingly, the primary purpose of this article is to analyze the influence of stewardship behavior dimensions on organizational citizenship behavior. The dimensions of stewardship behavior considered in this paper are Collectivist Orientation, Organizational Identification, Intrinsic Motivation, Involvement Orientation, Power Distance, and Use of Personal Power. Moreover, this paper also examines the mediating effects of idiosyncratic deals on stewardship behavior towards organizational citizenship behavior. This paper attempts to partially fill the research gap by using stewardship behavior as the antecedents of organizational citizenship behavior, which is mediated by idiosyncratic deals. Established on this rationale, the authors formulate and test a conceptual framework in which idiosyncratic deals mediates the link between stewardship behavior and organizational citizenship behavior.

Research Questions

The unreciprocated questions developed in this paper are:
What is the impact of stewardship behavior on organizational citizenship behavior?
What is the mediating effects of idiosyncratic deals on stewardship behavior towards organizational citizenship behavior?

Objectives

The above-identified questions are responded through the following objectives:
To analyze the influence of stewardship behavior on organizational citizenship behavior.
To examine the mediating effects of idiosyncratic deals on stewardship behavior towards organizational citizenship behavior.

Literature Review and Hypotheses

Stewardship Behavior: Gomez-Mejia et al. (2011) ascertained stewardship behavior as a indicator towards goal harmoniousness as it argues that directors gain ethical motive from pro-organisational and agglomerative behaviors that treasure altruism than separately self-motivated behaviors. Simms (2009) expressed stewardship behavior to

have farsightedness beyond the prevailing circumstances and achieve organisational goals by looking at the big picture of the organisation. Hernandez (2012) verbalized stewardship behavior as an authentic behavior with personal responsibility, moral action & courage, risk taking ability, and mental spirit to accomplish public affairs. Laszlo et al. (2003) pointed out the unselfishness of managers with stewardship behavior in their decisions and performance while attaining of organisational goals. Lubogoyi (2018) analyzed the relationship between perceived goal congruence and stewardship behavior using a sample of 310 participants working for local governments in Uganda. The results of structural equation modeling revealed a significant relationship between perceived goal congruence and stewardship behavior.

Idiosyncratic Deals: Guerrero and Challiol-Jeanblanc (2017) sought to design idiosyncratic deals as a fashion to nurture person perceptions of a positive employer image by extending customized extra subservient benefits. A survey among 182 engineers established a moderating effect of idiosyncratic deals on perceived external prestige towards turnover intention during the first years in the organization. Rousseau (2005) determined idiosyncratic deals as the negotiation between employees and the agents of employers at the time of engaging or while at work towards flexibility and role enhancement. Tang and Hornung (2015) conceptualized idiosyncratic deals engrafted in the contributions of work-family enrichment using 179 working parents in a Chinese city. The results of hierarchical linear regressions suggested that development idiosyncratic deals fertilized the intra-work role experience by tailor-making intrinsic work characteristics and thus increasing intrinsic motivation. Guerrero et al. (2016) investigated the antecedents and consequences of idiosyncratic deals considering career planning and career success. The results of a two-wave study among revealed a statistical significance between idiosyncratic deals and career success.

Organizational Citizenship Behavior: Yang and Wei (2018) elucidated the relationship between employee organizational citizenship behavior and ethical leadership. The authors also explained the moderating role of workplace ostracism on ethical leadership towards employee organizational citizenship behavior. The results of multiple regression analysis revealed a significant positive relationship between ethical leadership and employee organizational citizenship behavior. Ferris et al. (2009) addressed the research gap between job-limiting pain and political skill on job satisfaction and organizational citizenship behavior. The results of 237 respondents from South-eastern United States of America revealed that job satisfaction and organizational citizenship behavior decrease as job-limiting pain increase. Moideenkutty and Schmidt (2016) investigated the relationship among social exchange, liking, and supervisor-directed organizational citizenship behavior. The findings from 202 and 33 subordinates-supervisors dyad disclosed that social exchange did not mediate the relationship between liking and organizational citizenship behavior. Chung (2015) probed the mediating effects of organizational conflict on workplace ostracism towards organizational citizenship behaviors. The results of structural equation modeling showed full mediation of organizational conflict on workplace ostracism towards organizational citizenship behaviors.

Research Hypotheses: The research gap identified in this paper is evaluated using the following hypotheses:
H_1: Stewardship behavior dimensions will significantly influence organizational citizenship behavior.
H_2: Idiosyncratic deals will significantly mediate stewardship behavior towards organizational citizenship behavior.
Conceptual Framework

RESEARCH METHOD

Data were accumulated from spinning millings registered with South India Spinners Association. The respondents were employed as supervisors and managers. The primary data were gathered using two-wave cross-lagged self-reported questionnaires between November 2019 to February 2020. The first part (Time 1) of the questionnaire captured stewardship behavior and idiosyncratic deals. The second part (Time 2) of the questionnaire captured organizational citizenship behavior. There was a time gap of 3 months between two waves of the study. The self-reported questionnaires were disseminated to participants through their work e-mail with a cover letter. Overall,

self-reported questionnaires were sent to 1,426 employees at time 1 and 429 employees responded to the questionnaire (30.08%). At time 2, questionnaires were sent to 429 employees and 259 employees responded to the questionnaire (60.37%). A total of 226 responses were nailed down for further analysis after eliminating the unfinished responses. Pertaining to gender, 87% of the participants in this longitudinal sample were male. Regarding age, 39.1% were between 31–40 years of age. Concerning work experience, 42% of the respondents had employed in the company for 3–6 years. In addition, correlation, path analysis, and structural equation modeling were used to test the proposed hypotheses.

ANALYSES AND DISCUSSION

Objective 1: The influence of stewardship behavior on organizational citizenship behavior is analyzed using Karl Pearson's correlation. The dimensions of stewardship behavior: collectivist orientation, organizational identification, intrinsic motivation, involvement orientation, power distance, and use of personal power are tested against organizational citizenship behavior. It can be derived from the results of correlation coefficient analysis presented in Table 1 that there are significant positive correlations between organizational identification, collectivist orientation, and use of personal power and organizational citizenship behavior. It is also observed that there is a significant negative correlation between power distance and organizational citizenship behavior. Eventually, it was noticed that there are insignificant relationship between involvement orientation and intrinsic motivation and organizational citizenship behavior. More so, the maximum degree of correlation is observed between collectivist orientation and organizational citizenship behavior at 67.4%, which is followed by organizational identification and organizational citizenship behavior at 40.9%. Hence, H_1 is partially accepted.

Objective 2: The mediating effects of idiosyncratic deals on stewardship behavior towards organizational citizenship behavior is examined using path analysis in IBM AMOS. The path diagram is represented in Figure 2, and the mediating effects idiosyncratic deals on stewardship behavior towards organizational citizenship behavior is presented in Figure 3. It can be inferred from Table 2 that idiosyncratic deals do not mediate stewardship behavior towards organizational citizenship behavior (Halbesleben & Bellairs, 2016). Hence, H_2 is rejected. In addition, it is also observed that there is a significant relationship between organizational citizenship behavior and idiosyncratic deals.

Conceptual Framework: The factor loadings of the structural equation modeling is illustrated in Figure 4. The results of structural equation modeling disclose that the fit statistics (Hoyle, 2014; Kline, 2015) of the model fit the data adequately (2/df - 1.4775, NFI - 0.964, RFI - 0.969, CFI - 0.741 and RMSEA - 0.041). Hence, the overall fit of the proposed framework is established to be statistically vigorous (Ryu & West, 2009).

CONCLUSION, LIMITATIONS & FUTURE STUDY DIRECTIONS

Based on the findings and discussion in the previous subsection, the conclusions of this paper are as follows: An optimal organizational identification practices, collectivist orientation exercises, and ideal use of personal power can ameliorate organizational citizenship behavior among the spinning mill employees. In addition, minimal usage of power distance can significantly contribute organizational citizenship behavior of employees. The moderating of idiosyncratic deals do not significantly effect in relationship between stewardship behavior and organizational citizenship behavior. The primary research limitation lies in the data. In this research, our primary data were collected from a single source. Despite conducting two-wave longitudinal surveys, it is likely to be affected by common method bias. Therefore, the research findings should be taken with cautions. This paper also has authoritative practical implications. Motivating managers and supervisors to practice stewardship behavior is very significant because they have a direct impact on employees' extra-role behavior. This paper furnishes some executable direction for managers to achieve organizational citizenship behavior. Particularly, managers may use organizational citizenship behavior to motivate and support subordinates. In this study, the authors have discussed

only the negotiated deals in terms of idiosyncratic deal, future studies could focus on the impact of non-negotiated contracts (psychological contract) on other in-role and extra-role behaviors of employees.

REFERENCES

1. Anand, S., & Vidyarthi, P. (2015). Idiosyncratic deals in the context of Workgroups. *Idiosyncratic Deals Between Employees and Organizations*, 92-106. https://doi.org/10.4324/9781315771496-7
2. Chung, Y. W. (2015). The mediating effects of organizational conflict on the relationships between workplace ostracism with in-role behavior and organizational citizenship behavior. *International Journal of Conflict Management*, *26*(4), 366-385. https://doi.org/10.1108/ijcma-01-2014-0001
3. Ferris, G. R., Rogers, L. M., Blass, F. R., &Hochwarter, W. A. (2009). Interaction of job-limiting pain and political skill on job satisfaction and organizational citizenship behavior. *Journal of Managerial Psychology*, *24*(7), 584-608. https://doi.org/10.1108/02683940910989002
4. Gomez-Mejia, L. R., Cruz, C., Berrone, P., & De Castro, J. (2011). The bind that ties: Socioemotional wealth preservation in family firms. *Academy of Management Annals*, *5*(1), 653-707. https://doi.org/10.5465/19416520.2011.593320
5. Guerrero, S., & Challiol-Jeanblanc, H. (2017). Ex antei-deals, perceived external prestige and turnover intentions. *Personnel Review*, *46*(6), 1199-1212. https://doi.org/10.1108/pr-10-2015-0271
6. Guerrero, S., Jeanblanc, H., & Veilleux, M. (2016). Development idiosyncratic deals and career success. *Career Development International*, *21*(1), 19-30. https://doi.org/10.1108/cdi-08-2015-0115
7. Halbesleben, J., &Bellairs, T. (2016). What are the motives for employees to exhibit citizenship behavior?: A review of prosocial and instrumental predictors of organizational citizenship behavior. *Oxford Handbooks Online*. https://doi.org/10.1093/oxfordhb/9780190219000.013.16
8. Hernandez, M. (2012). Toward an understanding of the psychology of stewardship. *Academy of Management Review*, *37*(2), 172-193. https://doi.org/10.5465/amr.2010.0363
9. Hoyle, R. H. (2014). *Handbook of structural equation modeling*. Guilford Publications.
10. Kline, R. B. (2015). *Principles and practice of structural equation modeling* (4th ed.). Guilford Publications.
11. Kuppelwieser, V. G. (2011). Stewardship behavior and creativity. *management revu*, *22*(3), 274-295. https://doi.org/10.5771/0935-9915-2011-3-274
12. Lubogoyi, B., Kasekende, F., Kagaari, J., Ngoma, M., Munene, J. C., &Bakunda, G. (2018). Stewardship behaviour and perceived goal congruence in local governments in Uganda. *Leadership & Organization Development Journal*, *39*(6), 712-729. https://doi.org/10.1108/lodj-03-2018-0108
13. Moideenkutty, U., & Schmidt, S. (2016). undefined. *Review of International Business and Strategy*, *26*(2), 232-243. https://doi.org/10.1108/ribs-10-2013-0107
14. Nurjannah, H. (2019). Role of spirituality at work and organizational commitment to organizational citizenship behavior. https://doi.org/10.15405/epsbs.2019.05.02.12
15. Rousseau, D. (2015). *I-deals: Idiosyncratic deals employees bargain for themselves: Idiosyncratic deals employees bargain for themselves*. Routledge.
16. Ryu, E., & West, S. G. (2009). Level-specific evaluation of model fit in multilevel structural equation modeling. *Structural Equation Modeling: A Multidisciplinary Journal*, *16*(4), 583-601. https://doi.org/10.1080/10705510903203466
17. Simms, M. (2009). Insights from a management prophet: Mary Parker Follett on social entrepreneurship. *Business and Society Review*, *114*(3), 349-363. https://doi.org/10.1111/j.1467-8594.2009.00346.x
18. Tang, Y., & Hornung, S. (2015). Work-family enrichment through I-deals: Evidence from Chinese employees. *Journal of Managerial Psychology*, *30*(8), 940-954. https://doi.org/10.1108/jmp-02-2013-0064
19. Tihanyi, L., Johnson, R. A., Hoskisson, R. E., &Hitt, M. A. (2003). Institutional ownership differences and international diversification: The effects of boards of directors and technological opportunity. *Academy of Management Journal*, *46*(2), 195-211. https://doi.org/10.5465/30040614
20. Yang, Q., & Wei, H. (2018). The impact of ethical leadership on organizational citizenship behavior. *Leadership & Organization Development Journal*, *39*(1), 100-113. https://doi.org/10.1108/lodj-12-2016-0313

Table 1: Bivariate Correlation

Correlation		OI	CO	PD	IO	UP	IM	OCB
Organizational Identification	Pearson Correlation	1						
	Sig. (2-tailed)							
	N	226						
Collectivist Orientation	Pearson Correlation	.294**	1					
	Sig. (2-tailed)	.000						
	N	226	226					
Power Distance	Pearson Correlation	-.038	-.233**	1				
	Sig. (2-tailed)	.570	.000					
	N	226	226	226				
Involvement Orientation	Pearson Correlation	-.029	.242**	-.524**	1			
	Sig. (2-tailed)	.669	.000	.000				
	N	226	226	226	226			
Use of Personal Power	Pearson Correlation	.447**	.149*	-.006	-.060	1		
	Sig. (2-tailed)	.000	.025	.927	.368			
	N	226	226	226	226	226		
Intrinsic Motivation	Pearson Correlation	-.100	-.191**	.495**	-.576**	-.068	1	
	Sig. (2-tailed)	.134	.004	.000	.000	.308		
	N	226	226	226	226	226	226	
Organizational Citizenship Behavior	Pearson Correlation	.409**	.674**	-.173**	-.004	.168*	.106	1
	Sig. (2-tailed)	.000	.000	.009	.948	.011	.113	
	N	226	226	226	226	226	226	226

***. Correlation is significant at the 0.01 level (2-tailed).*

**. Correlation is significant at the 0.05 level (2-tailed).*

Table 2: Regression Weights

Regression Weights			Estimate	S.E.	C.R.	P	Label
Idiosyncratic Deals	<---	Stewardship Behavior	.218	.118	1.851	.064	Not Supported
Organizational Citizenship Behavior	<---	Idiosyncratic Deals	.156	.044	3.518	***	Supported

Source: Primary Data

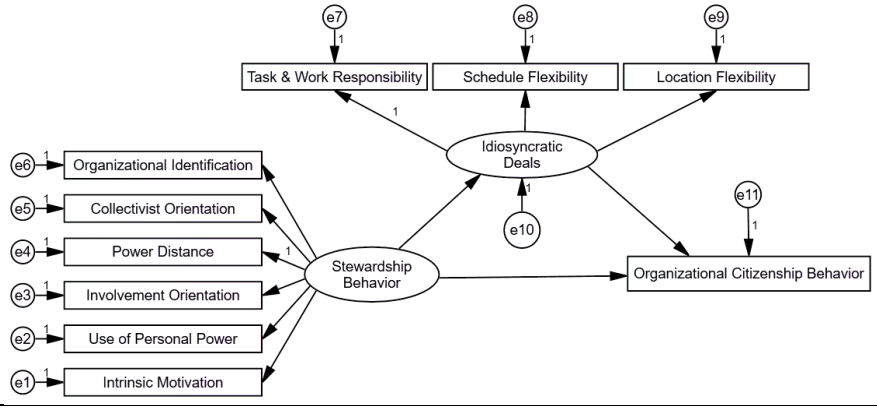

Figure 1: Conceptual Framework

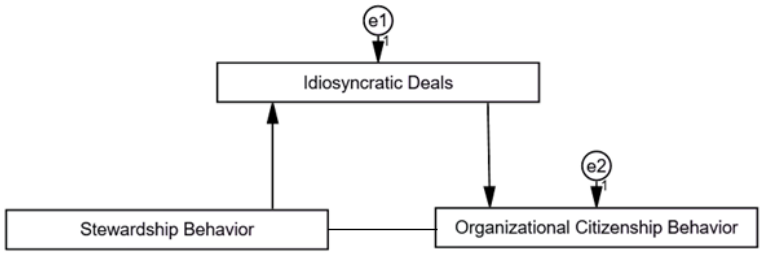

Figure 2: Path Diagram

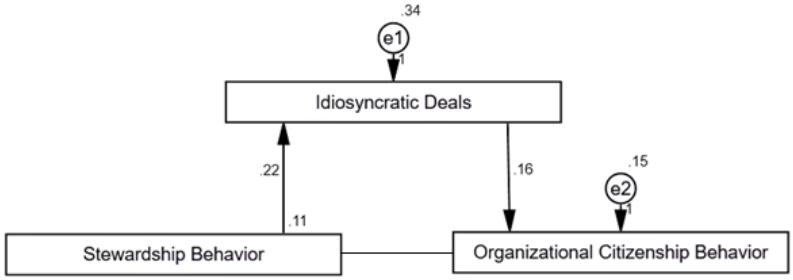

Figure 3: Mediating Effect

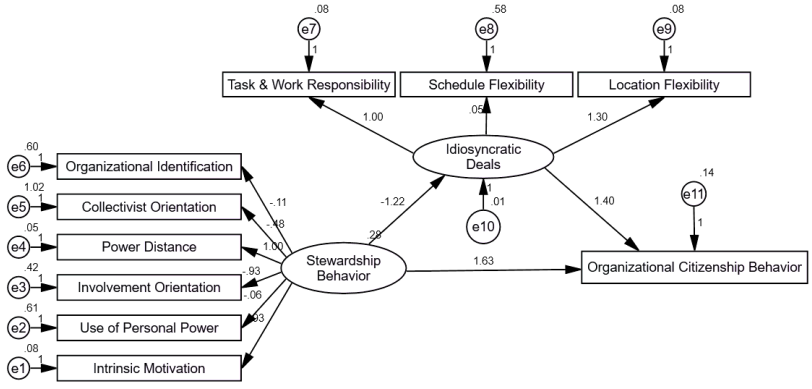

Figure 4: Conceptual Framework – SEM

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RESEARCH ARTICLE

Standardization of *Poora Satrasa Parpam*

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ABSTRACT

Siddha is an ancient system of medicine well practiced in South India. Drugs and cosmetic act 1940, Emphasizes standardization of ASU&H drugs. Scientific validation of safety and efficacy of the each and every Siddha formulation is mandatory before administration of drugs in humans. *Poora Satrasa Parpam* has not been standardized and clinically evaluated yet. Analytical study of the drug *Poora Satrasa Parpam* brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Analytical study includes many studies such as its Organoleptic properties, Physicochemical properties, and also to assess the active principles and elements present in the drug. Thus analytical study brings the efficacy and potency of the drug. As per PLIM Guidelines, the following analytical parameters were evaluated for *Poora Satrasa Parpam*. The results of *Poora Satrasa Parpam* is Confirm the standard for fineness as per the particle size analysis and flow property in water. Physico chemical, chemical and instrumental analysis indicates *Poora Satrasa Parpam* found in within the limits and as per the standard it prove the quality and it contains several compounds. Qualitative study of the drug ensures the purity of the drug. The Organoleptic characters, physicochemical and chemical values ensure the good quality and purity of the Siddha formulation *Poora Satrasa Parpam*.

Keywords: Siddha, Standardization, *Poora Satrasa Parpam*, Quality standards.

INTRODUCTION

Lord Shiva is considered to be the principal Siddhar and creator of Siddha system of medicine. Lord Shiva preached this grateful science to his wife Parvathi who is known as Shakthi, the goddess later Shakthi taught the science to

Nanthi. From nandhi the science was taught to Siddhars. Thus Siddha system was made available to common people. The Siddhars provided the norms for medicines that cure diseases as well as improve the health [10]. The diseases are initially treated by herbal medicines. If the diseases did not cured by herbal medicines then the medicines prepared from inorganic substances and animal products are used. So the inorganic substances and animal products could be used in Siddha system of medicine to treat the challenging disease and as well chronic diseases. In Siddha system 64 types of medicines have been identified and listed. They are divided into 32 types of Internal medicines and 32 types of External medicines [2].

Parpam is one among them. *Parpam* are the powder substances obtained by calcinations of purified metals, minerals and animal product with specific processes, commonly by *Pudam* (calcinations) process. Generally, this method of preparation of Siddha medicines involve conversion of minerals or metals or animal products into oxide or sulphide form by various herbal treatments followed by repeated high temperature calcinations or grinding cycles[10]. The *Parpam* thus obtained constitute ultra- small particles and are taken along with vehicles such as milk, honey, butter, ghee, etc. *Parpam* are white in colour. It is a compound infinitely superior marvelously potent and radically more effective than the herbal medical preparations such as *chooranam*(powder), *mathirai*(tablet), decoctions, syrup, medicated oils, medicated ghee, etc. In OPD and IPD of Ayothidoss Pandithar Hospital attached with National Institute of Siddha, many cases of patients are reporting with Peptic and Gastric ulcers with spasmodic abdomen. The drug *Poora Satrasa Parpam* is the one of the mineral preperation derived medicinal formulation mentioned in Anuboga Vaidhiya Navaneetham indicated for *Gunmam*(Peptic ulcer), *Soolai*(Spasmodic pain), *Vayvu*(painful conditioned), *Suram*(Fever) and *Sanni*(Delirium)[1]and this medicine is not evaluated in the aspect of Pharmacological.Hence the researcher chosen this drug to evalute the quality parameters in *Poora Satrasa Parpam.*

MATERIALS AND METHODS

*Poora Satrasa Parpam*is a Mineral Siddha formulation indicated for *Gunmam* (Peptic ulcer), *Soolai* (Spasmodic pain), *Vayvu*, *Suram*(Fever) and *Sanni*(Delirium)[1].Analytical study includes such as its Organoleptic properties, physicochemical properties, and also to assess the active principles and elements present in the drug have been studied [6]. Thus analytical study brings the efficacy and potency of the drug as per Pharmacopoeial Laboratory for Indian Medicine (PLIM) Guidelines.

Organoleptic Analysis

The organoleptic characters of the *Poora Satrasa Parpam* were evaluated. 1gm of the Test drug was taken and the Colour, Odour, Texture, Taste were seen (Table no.1).

Analysis as per classical Siddha literature

- Floating on Water (Figure no.1)
- Fine enough to enter the crevices of finger(Figure no.2)
- Irreversible reaction/ Solubility (Table no.2)
- Tasteless (Table no.3)
- Lusterless (Table no.3)

Physico Chemical Analysis

Physicochemical studies of the *Poora Satrasa Parpam* are essential for standardization, as it helps in determining the significance of physical and chemical properties of the substance being analysed and for the determination of purity and quality of the substance [4].The analysis includes the determination of ash value, Loss on drying at 105ºC,pH value, and extractive value[7]. These were carried out as per guidelines (Table no.4).

Chemical Analysis

The Qualitative analysis of Acid, Basic radicals and Miscellaneous constituents in it [5](Table no.5-8).

Instrumental Analysis

SEM (Scanning Electron Microscope): SEM analysis generates the signals which produce a two-dimensional image and reveals information about the sample. The information includes External morphological character, texture, crystalline structure, chemical composition and the shape and size of the particles of test drug[7] –*Poora Satrasa Parpam* was analysed through SEM as per standard procedure (Figure no.3).

EDAX: (Energy Dispersive X-Ray Analysis): The data produced by the EDX analysis consists of the spectra containing the elements present in the given sample (Graph no 1) which is being analysed [7]. It is also possible to get the elemental mapping and image analysis of the sample (Graph 1).

FT-IR (Fourier Transform Infra-Red): The FTIR instrument has globar and mercury vapour lamp as sources, an interferometer chamber comprising of KBr and mad Mylar beam splitters followed by a sample chamber and detector. Entire region of 400-4500cm – 1 is covered by this instrument. The spectrometer works under purged conditions. KBr or polyethylene pellets depending on the region of interest on that place Solid samples are dispersed. The typical resolution is 1.0 cm -1 cm. Signal averaging, signal enhancement, base line correction and other spectral manipulations are possible in this instrument [7].

Heavy Metal Analysis

Atomic absorption spectroscopy (AAS): As per the standard preparation of solution for the AAS, usually solution of 50 ml was prepared, in the proportion 1:25:25 ratio i.e., 1gm of sample were digested in 25 ml Con. Hcl and 25ml of Double distilled water and kept overnight and filtered the solution by Whatman filter paper, 50ml of prepared solution was added with 950ml of Double distilled water, finally 1000 ml solution was prepared which is used for the analysis purposes [7].The heavy metals of the *Poora Satrasa Parpam* were tabulated in (Table no.9).

RESULTS AND DISCUSSION

It was observed from the SEM analysis of *Poora Satrasa Parpam* that the average particle size of the sample ranges from 14.08 µm to 31.38 µm. From EDAX analysis, it was observed that, the drug *Poora Satrasa Parpam* has bio chemically important substances such as Oxygen (O), Sulphur (S), and Concentration of Mercury (Hg), Arsenic (As), Cadmium (Cd) and Lead (Pb) present in the drug was -0.02, 0.00, -0.01 and 0.02ug/g According to International Conference on Harmonisation [ICH], Permitted common concentration limits of elemental impurities, Mercury, Arsenic, Cadmium and Lead, across drug product component materials for products with daily intakes of not more than 10 grams are 3 ug/g, 1.5 ug/g. 0.5 ug/g and 0.5 ug/g On comparing the concentration of Hg, As, Cd and Pd in the drug with ICH permissible limits, though the drug *Poora Satrasa Parpam* has such toxic metals and minerals, they are within the ICH permissible limits can be considered to be safe for human consumption.

FTIR Results

In the FTIR Spectrum analysis, *Poora Satrasa Parpam* sample exhibits the peak value as shown in Table, at the wave number of 3506, 3506, 1658,1629,1350, 1138, 828, 707 having C=NH2 Stretch, N=H Stretch, C-N bending, O-H Stretch, O-H stretch, C-S bending, C=H bending, C-H bending. This indicates presence of some organic functional groups such as, conjugate anhydride, primary amide, carboxylic acid, secondary alcohols, alkene, alkane and halo compounds.

Heavy Metal analysis (AAS)

The Heavy metals like Arsenic, Mercury, Lead and Cadmium were found in within the normal limits.

CONCLUSION

It is concluded that Heavy metals like Arsenic, Mercury, Lead and Cadmium are present in Below Level of Quantification and the Presence of Trace elements such as Zinc, Iron, Calcium, Magnesium, Potassium in the *Poora Satrasa Parpam*. These are the essential for healthy human life and is substantiates the textual evidence of this formulation for the treatment of various disease conditions. Standardization is essential as a baseline studies for further research. It will pave the way for the Preclinical studies.

REFERENCES

1. Haikeemabdul sahib, *Anubogavaidhiyanavaneetham, part-4*, 2nd edition 2018, Thamarainoolagam, pg no 112.
2. Thiyagarajan.R, *Gunapadamthathujeevam*, Indian medicine &homeopathy, 8th edition,2013 pg no 282.
3. India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, 2014.
4. Pharmacopoeial Laboratory for Indian Medicine (PLIM) Guideline for standardization and evaluation of Indian medicine which include drugs of Ayurveda, Unani and Siddha systems, Department AYUSH, Ministry of Health & Family Welfare, Govt. of India.
5. Frank settle, Handbook of Instrumental techniques for analytical chemistry, ISBN 0-13-177338-0.
6. Ashotoshkar, New age international publishers, Pharmaceutical drug analysis 2nd Edition ISBN(978-91-224-2718-9).
7. Quality Control Standards For Certain Siddha Formulations (CCRAS),Editon1991.
8. Kannusamypillai C, Sikitcha Rathna Deepamennumvaidiyanool, Published by B.Rathina Nayakkar and sons, Vol 1, 3rd edition,1991.
9. Quality control Methods for Medicinal Plant Materials, WHO Geneva, 1998,P.28-33.
10. Narayanaswamy V, Introduction to Siddha System of Medicine, Tamil ValarchiKazhagam, first edition, 1975, Page no 68.
11. Balaramiah V, Greatness of Siddha Medicine, Saraswathy Sangam, Page no19.
12. Tripathi KD, Essentials of Medical Pharmacology,7thedition, Jaypee Brothers, Page no:859.
13. Advances in pharmacology & toxicology 2014, vol 15 issue 1,Page no 13-17.
14. European journal of pharmaceutical and medical research ISSN2394-3211.

Table No.1 Sample Description

State	Solid
Nature	Fine
Odour	Non- Characteristic / Odourless
Touch / Consistency	Soft
Flow Property	Free flowing
Appearance/ colour	Silver White

Table No. 2 Solubility Profile

S.No	Solvent Used	Solubility / Dispensability
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Insoluble
4	Ethyl acetate	Insoluble
5	DMSO	Soluble

Table No.3 Confirmatory Specification for *Poora Satrasa Parpam*.

S.No	Parameters	Observations for *PSP*
1.	Fineness	Confirms the standard for fineness as per the particle size analysis and flow property of the sample
2.	Float on Water	Confirms the test
3.	Smokeless	Confirms the test
4.	Taste less	Confirms the property
5.	Lustreless	Confirms the property

Table No.4 Ash values

S.No	Parameters	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	0.32 ± 0.11
2.	Total Ash (%)	92.03 ± 2.07
3.	Acid insoluble Ash (%)	0.44 ± 0.11
4.	Alcohol Soluble Extractive (%)	0.41 ± 0.02
5.	Water soluble Extractive (%)	9.06 ± 0.35

Table No.5 Chemical analysis of *Poora Satrasa Parpam*

S.No	Parameters	Observations	Results
1	Appearance of the sample	Silver White in colour	-
2	Solubility	Completely soluble	Absence of Silicate
3	Action on heat	No fumes evolved	Absence of carbonate
4	Flame test	No bluish green flame appeared	Absence of copper
5	Ash test	No yellow colour flame	Absence of sodium

Table No.6 Results for Acid radicals study of *PooraSatrasaParpam*

S.No	Parameters	Observations	Results
1	Test for Sulphate	Cloudy appearance present. A white precipitate insoluble in con.HCL was obtained	Positive
2	Test for Chloride	Cloudy appearance present	Positive
3	Test for phosphate	Cloudy appearance absent	Negative
4	Test for Carbonate	Cloudy appearance present	Positive
6	Test for Fluoride & Oxalate	No White Precipitate formed	Negative

Table No.7 Results for Basic radicals of *PooraSatrasaParpam*

S.No	Parameters	Observations	Results
1	Test for Lead	No Yellow precipitate formed.	Negative
2	Test for Aluminium	No Blue colour flame and precipitate formed	Negative
3	Test for Iron	Mild red colour appeared. Blood red colour appeared	Positive
4	Test for Zinc	White precipitate was formed	Positive
5	Test for Calcium	Cloudy appearance and white precipitate was obtained	Positive

6	Test for Magnesium	White precipitate was obtained	Positive
7	Test for Ammonium	Brown colour formed	Positive
8	Test for Potassium	Yellowish precipitate was obtained	Positive
9	Test for Mercury	No Yellow colour formed	Negative
10	Test for Arsenic	No Brownish colour formed	Negative

Table No.8 Results for Miscellaneous compounds of *Poora Satrasa Parpam*

S. No	Parameters	Observations	Results
1	Test for Starch	Blue colour developed	Positive
2	Test for reducing sugar	No discolourization was occurred	Negative
3	Test for alkaloids	Yellow colour developed	Positive
4	Test for Tannic acid	No Black colour formed	Negative
5	Test for type of compounds	No colour changes	Negative

Table No.9 AAS results of *Poora Satrasa Parpam*

S. No	Test parameters	Result	Unit
1	Arsenic (As)	BLQ (LOQ:0.01)	mg/kg
2	Mercury (Hg)	BLQ (LOQ:0.01)	mg/kg
3	Lead (Pb)	BLQ (LOQ:0.08)	mg/kg
4	Cadmium (Cd)	BLQ (LOQ:0.1)	mg/kg

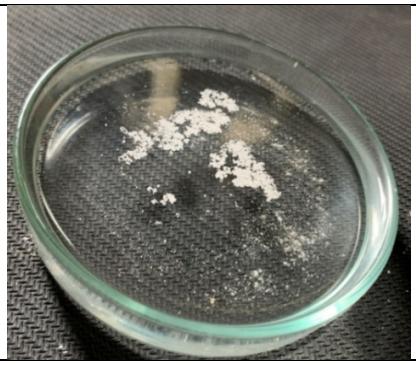


Figure 1: Float on water

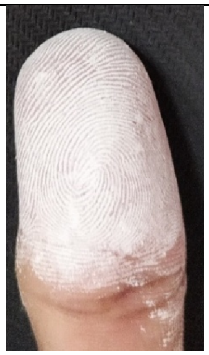


Figure 2: Fineness–Finger Ridge Deposit Analysis

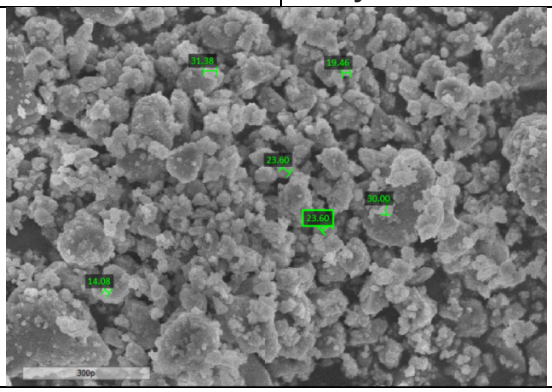
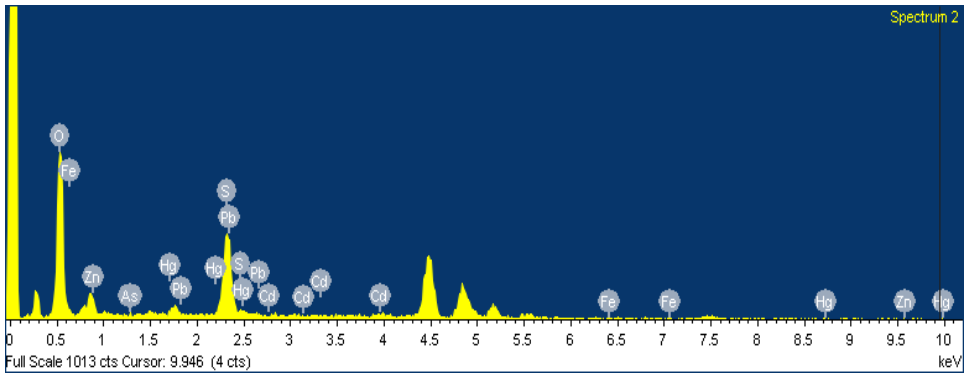


Figure 3: SEM Image of *PSP*



Graph 1 EDAX- Spectrum of *Poora Satrasa Parpam* – Cluster View

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REVIEW ARTICLE

Zoological Resources and Its Medical Importance in Siddha System of Medicine – A Review.

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ABSTRACT

Zoological resources play a vital role in Indian Medical systems particularly in Siddha medicine. In ancient time, Siddhars described about the importance and uses of various zoological species in their literatures which are mainly used for degenerative diseases, arthritis, respiratory disorders, infertility, skin diseases, etc. *Attai* (Leech), *Aamai* (Tortoise), *Udumbu* (monitor lizard), *Kilinjal* (Oyster shell), *Kombarakku* (Lac), *Kombu* (Horns), *Nandu* (Crab), *Poonagam* (Earth worm), Muttai odu (Egg shell), *Sangu* (Conch shell), *Palagarai* (Cypraea moneta) and *Maan kombu* (Deer horn) are commonly used in various formulations of Siddha medicines like *Parpam, Chenduram, Karukku, Kudineer*, etc. The present study is aimed to review the therapeutic uses of zoological resources and Siddha formulations with zoological species. In this review totally 45 zoological species and their parts have been used in more than 75 Siddha formulations. Most of the zoological ingredients based formulations have been indicated for *Moolam* (Piles), *Kaasam* (Tuberculosis), *Keelvaayu* (Osteo arthritis), *Chest pain* (Heart diseases), *Eraippu* (Bronchial asthma), *Maladu* (Infertility), *Odivu murivu* (Bone fracture), etc. Some preparations are specifically indicated for paediatric and geriatric diseases. These formulations are more effective, cost effective and easily available and can be prepared easily. This review study may help to initiate the in-depth biological researches to establish safe, efficacious, natural zoological ingredient based Siddha medicines with scientific validation for global acceptance.

Keywords: Zoological Resources; Siddha Medicine.

INTRODUCTION

Siddha system of medicine is an ancient traditional system of medicine developed by Sage Siddhars. For establishing this system, Siddhars adhere more on the natural resources for preparation of medicine and treating ailments. From time immemorial, the huge resources from plants, minerals, salts, metals and zoological were used for medicine preparation. There are 32 types of *Agamarundhu* (internal medicine) and 32 types of *Puramarunthu* (external medicine) followed by Siddhars and all the medicines used in Siddha are categorized under these types [1]. '*Ver paru, thazhai paru mella mella parpam chenduram pare*' is a famous quote by Siddhar. Hence according to this quote, the treatment regimen in Siddha falls on plant products as first line of treatment and then followed by metal, mineral and zoological medicines which include *parpam, chenduram, chunnam, kattu, kazhangu* etc [2]. These complex medicines are used by Siddha physicians to treat various chronic diseases like arthritis, anaemia, metabolic diseases, Diabetic mellitus, musculoskeletal disorders, auto immune diseases, deliberating diseases, cancer, etc.

Various parts of zoological sources were used extensively as potent ingredient in medicine formulations (Tab.no- 1, 2). For example feathers of birds are used for *Iragu parpam*, shell of snail used to prepare *Nathai parpam*, horns of deer used in *Sirungi parpam*, egg shell used in *Muttai oatu parpam*, conch shell used in *Sangu parpam*, earth worm used in *Poonaga chenduram*, shell of tortoise used in *Amai oatu karukku kudineer*, crab used in *Nandu theeneer*, human skull used in *Peranda parpam* [3]. As prophylactic measure, *Aalkaati patchi* egg for preventing measles, *Sirungi parpam* for preventing cardiac diseases, flesh of snail for preventing ano rectal diseases [1]. Moreover in Siddha, the Pearl (*Muthu*), Palagarai (*Cypraea moneta* Linn), *Sangu* (Conch shell) have been used extensively in practice. These have many medicinal values. As a traditional Chinese medicine (TCM), pearl powder has been widely used to treat palpitations, convulsions, epilepsy, eye diseases, and to promote wound healing [4-7]. Pearls have antioxidant properties [8] and provide high calcium bioavailability [9]. In addition, pearl powder has been used as a beauty treatment in China for thousands of years [8,10]. According to Siddha, Pearl has sweet and bitter taste with coolant nature and it has kozhaiyakatri (expectorant). *nachari* (antidote) , *kaamam perukki* (aphrodisiac), *udal uramakkai* (tonic), and isivakatri (anti spasmodic/ anti epileptic) actions. Pearl (*Muthu*) prepared as pearl ash known in Siddha literature as *Muthu parpam*, is traditionally used in Siddha to treat many diseases. *Muthu parpam* is useful for all types of *suram* (fever), *kapha* diseases (respiratory disorders), *vidangal* (toxic conditions), *nethra noikal* (eye diseases) and *inthiriya nattam* (enhance the quality of semen) [1].

Natural pearls from the oyster *Pteria martensii* (Dunker) occur in sea water, and mainly grow in Hainan and Guangxi provinces as a precious medicine with a high price. They are typically used as a health food supplement during pregnancy and the postpartum period [11]. The marine mineral drugs, including pearls, nacre, clam shell, common oyster shell, ark shell, sea-ear shell, and cuttle bone, have special qualities of both mineral drugs and animal drugs. The chemical composition of these materials is mainly calcium carbonate (CaCO3), accounting for about 90% of the weight, followed by conchiolin protein, and a small amount of trace elements [12,13]. Conch is a common name that is applied to a number of medium to large-sized shells of large snails (*Turbinella pyrum*) from the family Turbinellidae [14]. Structurally, conch is a porcelaneous shell of an oblong or conical form with bulging in the middle and tapering at each end [15]. Conch (Sangu) prepared as conch shell ash, known in Siddha literature as *Sangu parpam*, is used to treat various ailments. According to Siddha, conch has *udal uramakki* (alterative), *pasithe thoondi* (stamachic), *thuvarppi* (astringent), *veppakatri* (febrifuge), *thuyarakki* (anodyne), *akattuvayuvakatri* (carminative) *nachari* (detoxifying) and *kazhaiakatri* (expectorant) properties. It is useful for hyperacidity (Gunmam), loss of appetite (*seriyamai*), irritable bowel syndrome (*kirakhani*), jaundice (*kaamalai*) pain in the abdomen (*akattu vaayu*), hypertension (*rathapiththam*), eye diseases (*nethra noikal*) and more useful to reduce the menstrual pain (*soothaga sanni*) when administered with suitable vehicle (*anubanam*) within the maximum therapeutic dose of 500 mg per dose. It is also used as external application for warts (*maru*), pimples (*mugaparu*) and enhances the hair growth. (1) Recent research on incinerated conch has focused on pharmaceutical standardization of compressed tablets along with estimation of calcium content and acid neutralization capacity as an antacid [16]. It has also shown antispasmodic effect on

acetylcholine induced excised rat ileum compared to atropine, therapeutic response to acetic acid induced writhing test in rats and in vitro anti-inflammatory activity related to inhibition of protein denaturation [17]. It also showed in vivo dose dependent protection against gastric ulcer and anti-peroxidative effect without altering serum calcium level, but additional mucin production as compared to standard Ranitidine [18]. *Anupanam* (Adjuvant) used in Siddha is a unique approach to treat various diseases with a single medicine. *Anupanam* acts as catalysts to enhance the efficacy of the medicine to reach the target organ with added benefits. Many zoological resources are used as *anupanam*. Cow milk, goat milk, donkey milk, honey, fat of animals, ghee, butter, buttermilk, egg white, egg yolk, etc are commonly used as *anupanam* for various medicines with their significant role in treating the diseases [1]. According to the World Health Organization, about 70–80% of the world populations inclines on traditional systems for their improving health and as remedies for diseases [19]. In this scenario, the Indian systems of medicine like Siddha, Ayurvedha , Unani are being an emerging source of medical system through various evidence based studies have proved their own strength and efficacies. In the current situation, though there are increasing researches in the traditional systems, the use of animal resources has been decreased due to restrictions and availability of these resources and hence in the same way, the researches in this area are inclined. The huge utilisation of zoological resources as medicine in Siddha has to be validated through various analytical, *in-vitro* and animal studies in future. This review is aimed to give a collective idea about various animal resources and their peculiarity and importance in Siddha for further research.

METHODOLOGY

The traditional Siddha books from the library of National Institute of Siddha were reviewed to collect the information of zoological resources used in Siddha and the databases like Pubmed, Google scholar were used to retrieve scientific information for the zoological resources used in Siddha.

RESULTS AND DISCUSSION

The details of zoological resources used in Siddha and their scientific names were listed in Table 1. Details of medicines prepared from zoological resources and their medicinal uses were listed in Table 2. As per the information obtained, there are huge varieties of zoological resources extensively used to alleviate the diseases with simpler formulations. The zoological resources are widely used in medicine preparations, purification of drugs, as adjuvant and diets for diseases. These resources can be used for all ages from infants to geriatric population without any adverse effect for eg. *Amai ottu karuku kudineer* and *Mulleli chooranam* are especially used for children, *Peranda parpam* for Alzhiemer diseases in old age people. Hence this wide array of medicinal uses of these zoological resources can be utilised for the flourishing research in this area.

CONCLUSION

Zoological resource plays an important role in medical practice. The usage mentioned in ancient Siddha literatures has to be reviewed for scientific validation and global acceptance. More clinical studies have to be conducted to reassure the facts claimed in Siddha literatures.

REFERENCES

1. Thiyagarajan R, “Gunapadam – Thathu Jeeva vakuppu”, 2nd edition, 2006,published by Directorate of Indian Medicine and Homeopathy, Chennai-106,
2. Eugene Wilson, GV. Rajamanickam, Neera Viyas, A.Agarwal, GP Dubey, Herbs used in Siddha medicine for arthritis- A review, Indian Journal of Traditional Knowledge,2007;Vol 6 (4),pp-678-686.

3. Kuppusamy Mudhaliyaar K N & Uthamaraayan K S, "Siddha Vaidhiya Thirattu", (Indian Medicine and Homeopathy Department Chennai) 2009.
4. China Pharmacopoeia Committee, Pharmacopoeia of the People's Republic of China, China Chemical Industry Press, Beijing 2010.
5. Dai, J. P., Chen, J., Bei, Y. F., Han, B. X., Guo, S. B., Jiang, L. L., Pharm. Biol. 2010, 48, 122–127.
6. Xu, H. B., Huang, K. X., Gao, Q. H., Gao, Z. H., Han, X. X., Pharmacol. Res. 2001, 44, 1–6.
7. Shen, Y. T., Zhu, J., Zhang, H. B., Zhao, F., Biomaterials 2006, 27, 281–287.
8. Shao, D. Z., Wang, C. K., Hwang, H. J., Hung, C. H., Chen, Y. W., J. Cosmet. Sci. 2010, 61, 133–145.
9. Chen, H. S., Chang, J. H., Wu, J. S. B., J. Food Sci. 2008, 73, 246–251.
10. Zhang, L. H., Wang, J. L., Mei, H. P., Luo, B., Deterg. Cosmet. 2002, 25, 177–180.
11. Chuang, C. H., Chang, P. J., Hsieh, W. S., Tsai, Y. J., Lin, S. J., Chen, P. C., Int. J. Nurs. Stud. 2009, 46, 787–795.
12. Yan, G. M., Li, X. L., Zeng, S., Sun, J. Y., Res. Pract. Chin. Med. 2009, 23, 20–22
13. Wei, Z., Chen, H. P., Chen, L., Peng, Y., Yin, L., Pharm. Clin. Chin. Mater. Med. 2012, 3, 10–13.
14. Wikipedia. Conch. Available online: https://en.wikipedia.org/wiki/Conch (accessed on 10 September 2018).
15. Nadkarni, K.M.; Nadkarni, A.K. Indian Materia Medica, 3rd ed.; Popular Prakashan Private Ltd.: Mumbai, India, 1954; Volume 2, pp. 164–165, ISBN 81-7154-142-9
16. Seth, A.; Maurya, S.K.; Shrivastava, A. Formulation development, characterization and estimation of acid neutralizing capacity of Shankha Bhasma tablets for the treatment of dyspepsia. Int. J. Pharm. Pharm. Sci. 2014, 6, 467–469
17. Thakur, V.; Vashisht, K.; Sharma, K. Therapeutic indications of Shankha Bhasma: A review. Int. Res. J. Pharm. 2017, 8, 1–6. [CrossRef].
18. Pandit, S.; Sur, T.K.; Jana, U.; Bhattacharya, D.; Debnath, P.K. Anti-ulcer effect of Shankha Bhasma in rats: A preliminary Study. Indian J. Pharmacol. 2000, 32, 378–380.
19. Jacqui W. Herbal products are often contaminated, study finds. *BMJ.* 2013;347: f6138.

Table .1. Name of the Zoological resources used in Siddha system of Medicine

S.No	Siddha (Tamil) Name	English Name	Zoological Name
1.	*Attai*	Leech	*Hirudo medicinalis*
2.	*Aamai*	Tortoise	*Chelonia turtle*
3.	*Inthirakoba Poochi*	Red velvet ant	*Mutilla occidentalis*
4.	*Iragukal*	Feathers of birds	-
5.	*Udumbu*	Monitor	*Varanus varius*
6.	*Eri vandu*	Telini fly	*Mylabris sps*
7.	*Elumbukal*	Bones of animals	-
8.	*Kasthuri*	Musk	***Mosc***hus moschiferus
9.	*Kandamiruka kombu*	Horn	*Rhinoceros unicornis*
10.	*Kilinjal*	Oyster shell	*Ostrea edults*
11.	*Kuzhambukal*	Nails of animals	-
12.	*Kobarakku*	Lac	*Carteria lacca*
13.	*Korosanam*	Ox Gall	*Bos Taurus*
14.	*Kozhi*	Cock, Hen	*Gallus domesticus*
15.	*Sangu*	Conch	*Turbinella rapa*
16.	*Suraa*	Shark	*Squalus carcharius*
17.	*Thantham*	Teeth of animals	-
18.	*Thean*	Honey/ Mel	-
19.	*Nandu*	Crab	*Cancer pagurus* Linn
20.	*Naththai*	Snail	*Helix pomatia*

21.	*Nari echcham*	Jackal's excreta	*Canis aureus*
22.	*Palagarai*	Cowry	*Cypraea moneta* Linn
23.	*Pantri*	Pig	*Sus scrofa cristatus*
24.	*Paal*	Milk of animals	-
25.	*Pichchi*	Bile of animals	-
26.	*Puli*	Tiger	*Panthera tigris*
27.	*Puraa echcham*	Dove	*Columbidae*
28.	*Saanam*	Dung of animals	-
29.	*Punugu*	Civet cat	*Viverra civetta*
30.	*Poonagam*	Earth worm	*Lumbricus terrestris*
31.	*Mail*	Peacock	*Pavo cristatus*
32.	*Maan*	Deer	*Cervidae*
33.	*Minminip poochi*	Fire fly	*Lampyridae*
34.	*Musurumuttai*	Red ant egg	*Pogonomyrmex barbatus*
35.	*Muttai odu*	Egg shell of birds	-
36.	*Muthalai*	Crocodile	*Crocodilus porosus*
37.	*Muththuchippi*	Oyster shell	*Mytilus margaritiferus*
38.	*Mezhugu*	Wax	-
39.	*Yanai thantha*	Elephant	*Elephas indicas*
40.	*Kuzhavik koondu*	Wasps nest	*Vespula germanica*
41.	*Kombukal*	Horns of animal	-
42.	*Siruneer*	Urine of animals	-
43.	*Pambu sattai*	Snake's slough	-
44.	*Mulleli*	Hedgehog	*European hedgehog*
45.	*Oanan*	Calotis	*Agama agama*

Table 2. Name of the Zoological medicinal formulations used in Siddha medicine

S.No	Name of the Siddha preparation	Medicinal Uses
1	*Aamai ottu karukkuk kudineer*	*Seriyamai* (indigestion), *Vairu porumal* (abdominal discomfort).
2	*Aamai ottu karukku*	*Seriyamai* (indigestion), *Vairu porumal* (abdominal discomfort), *Kapha noikal* (respiratory syndrome), *Pedhi* (diarrhoea),*Vanthi* (vomiting)
3	*Aamai ottu parpam*	*Pedhi, Vairu porumal* (Paediatric diarrhoea and abdominal discomfort), *Seriyamai* (indigestion).
4	*Aamai legium*	*Moolam* (Piles), *Udal thetri* (general tonic).
5	*Sarva vidari kuligai*	*Pambu kadi vidam* (Snake bite) , *Vandu kadi* (insect bites)
6	*Udumbu chooranam*	*Pedhi* (Diarrhoeal diseases), *Moolam* (piles), *Soothaga vali* (painful menstruation)
7	*Udumbu legium*	*Udal thetri* (General and nervine tonic)
8	*Udumbu nei*	*Raththaperukku* (All kind of bleeding disorders)
9	*Kottan elumbu Parpam*	*Karuppai kattikal* (Cystic condition in reproductive organs)
10	*Peranda parpam*	*Mana noikal* (Mental disorder)
11	*Kasthuri mathirai*	*Peenisam* (Sinusitis), *Suram* (fever), *Kabala soolai* (headache).
12	*Siru kilinjal parpam*	*Madhumegam* (Diabetes), *Kapha noikal* (respiratory symptoms)
13	*Kilinjal mezhugu*	*Pithavedippu* (heel fissure)
14	*Kaal kuzhambu parpam*	*Thiridhosam, vatha noikal*(arthritis)

15	*Kuzhavik koondu*	*Vikkal* (hiccough)
16	*Arakku thylam*	*Kabalasoolai* (head ache), *Peenisam* (sinusitis), *Theka kaduppu* (body ache)
17	*Kombarakku chooranam*	*Rathapokku* (bleeding disorders)
18	*Kombu parpam*	*Unmaatham* (mental disorders), *Kalladaippu* (stone disease), *Thummal* (sneezing), *Irumal* (cough)
19	*Mathana Kamesuram*	*Thathhu nattam* (increase semen volume, sperm count and motility), semen enhancer.
20	*Korosanai ennai*	*Acharam* (oral ulcer)
21	*Kozhikkal*	General tonic, appetizer
22	*Muttai ottu parpam*	*Suram* (fever) and its complications
23	*Andathylam*	*Pechu kuzharal* , *Naa vatham* **(**dysarthria)
24	*Sitranda Mezhugu*	*Soolai* (pricking painful condition)
25	*Anda erukkan Jayaneer*	Precursor for higher order siddha medicine preparation
26	*Karunkozhi Chooranam*	*Venkuttam* (leocoderma), *Moolam* (piles), *Vandukadi* (insect bites)
27	*Sangu parpam*	*Gunam* (gastritis), *seriyamai* (loss of appetite), *kirakhani* (irritable bowel syndrome), *kaamalai* (jaundice), *akattu vaayu* (pain in the abdomen).
28	*Sangu Chenduram*	*Pithathikkam* (diseases caused deranged pitha humour), *Paandu* (anaemia), *Venkuttam* (leucoderma)
29	*Vellai Mathirai*	*Kan padalam* (pterygium)
30	*Saana patru*	*Veekkam* (edema caused by trauma)
31	*Cow's urine*	*Paandu* (anaemis), *Sobai* (fluid accumulation), *Kaamali* (jaundice)
32	*Sura puttu*	*Gunmam* (gastritis), *Athisaaram* (diarrhoea),
33	*Sura nei*	*Kanai* (primary complex), burns
34	*Thantha parpam*	*Mahotharam* (ascites), *Vaatha noikal* (musculo skeletal disorder), *Piramai* (psychological disorders), *Athisaaram* (diarrhoea), *Madhumegam* (diabetes).
35	*Then karkandu*	*Kan padalam* (pterygium), *Suram* (fever)
36	*Nandu theeneer*	*Shayam* (tuberculosis), *Kulir suram* (fever with rigor)
37	*Nandu kozhuppu*	Used for higher order siddha medicine preparation
38	*Nanducharu*	*Neerkorvai* (sinusitis/ rhinitis), *Thekakaduppu* (body ache).
39	*Nandu Kuzhineer*	*Vanthi* (nausea, vomiting) , *Thaagam* (excessive thirst), *Vikkal* (hiccough)
40	*Nandu kuzhambu*	*Shayam* (tuberculosis)
41	*Nathaisippi parpam*	*Raththa moolam* (bleeding piles), *Seetha bedhi* (dysentery)
42	*Thaalaga karuppu*	*Kaasam* (bronchial asthma), *Irumal* (cough), *Suram* (fever)
43	*Nathai legium*	*Asana noikal* (ano rectal diseases)
44	*Nari echham Pugai*	*Pakanthram* (fistula in ano), *Moolam* (piles)
45	*Palagarai parpam*	*Nanjunilai* (toxic conditions), *Kaanakadi* (utricaria), *Peenisam* (sinusitis), *kaayam* (wounds),
46	*Palagarai chenduram*	*Kapha disorders* (respiratory diseases), *Ruthra vaayu* (cardiac diseases)
47	*Palakarai kalimbu*	*Punundakki* (caustic)
48	*Pantri nei*	*Ratha moolam* (bleeding piles), *Theeppun* (burns), *Neerkaduppu*

		(burning micturition)
49	*Silai thylam*	*Puraiyodiya viranam* (cancerous ulcer)
50	*Paamnbu sattai thylam*	*Paithiyam* (psychological disorder)
51	*Saranathi thylam*	*Kabala soolai* (head ache)
52	*Navaneetha parpam*	*Gunmam* (gastritis), *Moolam* (piles), *Maaradaippu* (heart disease)
53	*Kaatu seeraka chooranam*	*Venkuttam* (leucoderma)
54	*Pulinaga parpam*	*Thrithosam*
55	*Pulinaga ennai*	*Mugaparu* (pimples)
56	*Kasthuri mezhugu*	*Nadukku vatham* (Parkinson's disease), Maaradaippy (heart disease), Suram (fever), Soothaga sanni (dysmenorrhea)
57	*Poonaga karukku kudineer*	*Naavaratchi* (excessive thirst), *Sanni thodam* (delirium)
58	*Poonaga karukku*	*Naavaratchi* (excessive thirst), *Sanni thodam* (delirium)
59	*Poonaga chaththu*	Synthesis of bio-copper and used for higher order siddha medicine preparation
60	*Poonaga chenduram*	*Shayasuram* (fever due to lung infection)
61	*Mailiraku parpam*	*Vikkal* (hiccough), *Moochadaippu* (breathing difficulty)
62	*Mail elumbu parpam*	*Venkuttam* (leucoderma)
63	*Mail muttai ottu parpam*	*Vettai rogam* (Venereal diseases)
64	*Sirunki parpam*	*Thamaraga noikal* (heart diseases)
65	*Musuru muttai ennai*	*Suram* (fever), *Sanni* (delirium), *Vatham* (muscular skeletal disorder)
66	*Muttai ottu parpam*	*Kaasam* (bronchial asthma), *Maaradaippu* (cardiac diseases), *kapha rogam* (repiratory disorder)
67	*Muthuchippi parpam*	*Kapha dhosam*(respiratory disorder), *Keelvaayu* (arthritis)
68	*Mezhugu kalimbu*	*Viranam* (wounds)
69	*Mezhugu thylam*	*Pakkavatham* (hemeplegia),Vettukaayam (injury), *Keel vaayu* (arthritis)
70	*Pinda thylam*	*Vatha noikal* (musculo-skeletal disease), *Pithavedippu* (heel fissure)
71	*Chandrakala lebam*	*Neerkorvai* (sinusitis), *Kabalasoolai* (head ache)
72	*Mulleli Chooranam*	*Kanai* (primary complex), *Irumal* (cough)
75	*Mulleli thylam*	*Eraippu* (bronchial asthma)

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RESEARCH ARTICLE

Molecular Docking Studies of Phytocompounds against C-Reactive Protein- 1LJ7 of Colon Cancer

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ABSTRACT

Colon cancer is the third most commonly occurring cancer in men and the second most occurring cancer in women. Though there are drugs and treatments for colon cancer, the death rate is over 8.8 lakhs in India (NCI-AIIMS). Thus, there is a need to develop more natural drug for colon cancer. *Tamarindus indica* L. is a multipurpose evergreen tree and an important cash crop of India which is used for its fruits in food industry. Tamarind was recorded as a variable tree species over a century ago especially for its pulp colour, sweetness and pod production. The fruit pulp contains minerals, polyphenols and tartaric acid. Apart from its domestic and industrial applications, it has a wide range of medicinal values- antimicrobial, antidiabetic and antiulcer. Tamarind pulp which has a natural anticancer property with potential phytocompounds (Squalene, Gamma-sitosterol, 3-Hydroxy-2,3-dihydromaltol) analysed by GC-MS and is retrieved from pubchem database and docked against the target protein (C- reactive protein which causes inflammation at the early stage of colon cancer) which is retrieved from Protein Data Bank (PDB) and compared with the standard drug (Leucovorin calcium). Both ligand and target are docked using PatchDock software. Results revealed that squalene has a higher docking score (5266) when compared with the standard (4668) and other two compounds (4318 and 3130) respectively. Thus, Squalene- a triterpenoid is found to be an effective inhibitory compound which reduces the inflammation caused by C-reactive protein which in turn minimizes the frequency or incidence of colon cancer. Thus, an appropriate amount of Tamarind pulp intake in our diet might reduce or prevent the possibility of

colon cancer and in vivo studies will be carried out by isolating the specific compound from Tamarind pulp for further investigations.

Keywords: Tamarind pulp, GC-MS, Squalene, C-reactive protein, Colon cancer, Docking.

INTRODUCTION

Colon cancer is common in both men and women [1]. It is the main cause for morbidity and mortality throughout the world [2], where almost 9% of all cancer incidences take place [3]. The death rate is estimated to be over 8.8 lakhs in India [4]. The era we live in has an improved worldwide average living and increased access to adequate healthcare which leads to the considerable improvement in the diagnosis and treatments to diseases [5]. Though there are several drugs and treatments for colon cancer, the death rate is higher and there is need to develop more specific and natural drug for colon cancer. *Tamarindus indica* L. is a multipurpose evergreen tree used mainly for its culinary value of fruits and an important cash crop of India, popularly known as 'Indian Date' [6] which belongs to the family Fabaceae and it is the third largest family of flowering plants [7]. India ranks 6th position in export of Tamarind with an average production of about 1,91,750 tonnes [6]. In India, Tamil Nadu ranks first in the production of tamarind fruit with an average production of 44.55 tonnes and are widely cultivated in Dindigul, Theni, Madurai and Trichy with preferred varieties of PKM1 and Urigam [8].

Tamarindus is a monotypic genus that contains a sole species *T. Indica.* It is a perennial tree with a thick upright trunk and bright green foliage. Flowers are protogynous, entomophilous and largely cross- pollinated. The fruits are pods which are generally ripen about 10 months after flowering; it has an outer pericarp and inner pulp. The pulp has seed cavities in which the seeds are present and the seeds are orthodox which can be stored up to 2 years [9]. Trees start bearing fruit after 7- 10 years with a maximum yield in 15 years and continue to yield for 200 years. A well managed tree can yield from 300- 500 kg of ripe pods and tree can survive up to 300 years [10]. Tamarind is an underutilized tree crop and therefore an under researched species but the fruit contains important source of mineral elements and the polyphenols present in tamarind pulp have high antioxidant and anti-inflammatory property which protect against cardiac problems, cancer and diabetes. Apart from medicinal use, it has a wide range of domestic and industrial applications [6]. Therefore, the objective of the study is to qualitatively screen the phytochemicals present in Tamarind pulp using aqueous and methanol extracts followed by GC-MS analysis to characterize the bioactive compounds and further *in silico* work has been carried out to evaluate the functional role of the selected potential phytocompounds.

MATERIALS AND METHODS

Collection of Tamarind Pods

Tamarind pods (fresh) were collected based on various geographical locations (sample-1, sample-2, and sample-3). The outer shell, fibre and seeds were removed manually. The fresh Tamarind pulp was stored in an air tight container, later used for various phytochemical and GC-MS analysis.

Crude extract preparation

The crude extract was prepared using 10 gm of the fresh tamarind pulp dissolved in 100 mL of solvents (methanol and aqueous), kept at room temperature for 24 hrs after which the extract was filtered and used for further analysis.

Phytochemical screening

The aqueous and methanol extracts of three samples (sample-1, sample-2, sample-3) of fresh Tamarind pulp were tested qualitatively for the identification of alkaloids, phenols, terpenoids, tannins, flavonoids, glycosides, saponins, carbohydrates, proteins and amino acids using standard procedures [11].

GC-MS analysis

One ml methanol extract of fresh Tamarind pulp of three samples (sample-1, sample-2, sample-3) was used and the GC-MS analysis was performed in VIT University, Vellore using the Clarus 680 GC packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df). Helium was used as a carrier gas with a constant flow rate of 1 ml/min. One μL of sample extract was injected into the instrument with the following parameters, initial oven temperature 60°C for 2 min; followed by an increase of 10°C min^{-1} up to 300 °C and was held for 6 min. The components obtained from the spectrum were compared with the known components of the database spectrum stored in the GC-MS NIST (2008) library.

In silico analysis (Docking studies)

In silico analysis through molecular docking plays a vital role in identifying the functional role of a particular bioactive compound. The compounds were screened by GC-MS analysis in the Tamarind pulp of three samples collected from various geographical locations were screened against the target protein (C - reactive protein) in order to study the anti-cancer property of Tamarind pulp against colon cancer. The target protein (PDB ID: 1LJ7) was retrieved from (PDB) Protein Data Bank (http: //www. rcsb. org/pdb/) where as the bioactive compounds were retrieved from the Pubchem database and were docked against the target protein using bioinformatic tools. Docking studies were carried out to assess the anti-cancer property of the phytocompound present in Tamarind pulp. Leucovorin calcium was used as a standard for colon cancer.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical analysis carried out in Tamarind pulp using methanol and aqueous extracts had revealed the presence of phytoconstituents which were found to be similar for all the three samples collected from various geographical locations. The fresh Tamarind pulp indicated the presence of phenols, terpenoids, tannins, flavonoids, glycosides, saponins, carbohydrates and amino acids [table 1]. The chemical constituents present in the plants are said to have a biologically important active compounds and are known as secondary metabolites [12]. The primary function of a plant metabolite is to protect the plant against pathogens and other environmental factors like pollution, stress drought etc [13]. The novel phytoconstituent's mainly secondary metabolites are directly involved in improving human health by acting as an antioxidant and having anti-cancer property [14].

In traditional medicine, the Tamarind pulp is used in abdominal pain, diarrhoea and wound healing. It is used as laxative because of its higher constituents of tartaric acid, malic acid and potassium content. In Thailand, the Tamarind pulp is available commercially in the form of tablet for the reduction of excess weight. One of the compounds named xyloglucan from Tamarind pulp is used in eye surgery for the conjuctival cell adhesion and corneal wound healing. The fruit extracts have shown to improve the ibuprofen effect in humans. It also prevents LDL-Cholesterol which is the main cause for arthrosclerosis. The presence of saponins in tamarind pulp is said to have molluscicidal activity [15]. Steroids are mainly used in skin inflammation and asthma [16]. The terpenoids which has high antioxidant potential acts as an anti inflammatory and anti-cancer activity [17, 18]**.** In addition, the presence of tannins and flavonoids in tamarind pulp enhances the anti-cancer property [15]. The presence of these phytocompounds in Tamarind pulp can be a potential source for medicinal use.

GC-MS analysis

GC-MS is an analytical technique with a combination of Gas chromatography (GC) and Mass spectrometry (MS) used to analyse the potent bioactive compounds in a biochemical mixture [19]. The GC-MS analysis of Tamarind pulp collected from various locations (sample-1, sample-2, and sample-3) were carried out using methanol extract. The chromatogram for sample-1 showed the presence of 20 compounds, of which 6 compounds lie in a good probability range. Levanoglucosenone,-α-d-Glucopyranoside, α -D-fructofuranosyl (preservative), palmitic acid

(lubricant, hemolytic, pesticide, anti androgenic, antioxidant, flavor, α reductase inhibitor, hypocholsteremic,), 9,12-Octadecadienoic acid (anti-cancer) squalene (antibacterial, anti ageing, analgesic, vasodilator, anti-diabetic, anti ulcerogenic, anti-inflammatory, hypocholesterolemic, anti spasmodic, anti-bronchitic, anti coronary antioxidant anti-tumor, immune stimulant, chemo preventive, lipoxygenase-inhibitor pesticide) are the major bioactive compounds in the fruit pulp. The gas chromatogram of sample-2 showed 23 compounds of which 5 compounds were showing higher peak percentage (>50%). They are 5-Hydroxymethyl-2-furan carboxyaldehyde, 2-Butoxyethyl acetate, (preservative), Nanadec-l-ene, n-Tetracosan-l-ol, Gamma-Sitosterol (anti tumour property)

The gas chromatogram of sample-3 showed 23 compounds of which 8 compounds were in higher range of peak percentage (>50%). They are furfural, 2-Furancarboxaldehyde, 5-methyl-, 2,4-Dihydroxy-2,5-dimethyl-3[2H]-furan-3-one, 5-Hydroxymethyl fural (preservative) palmitic acid (lubricant, hemolytic, pesticide, anti androgenic, antioxidant, flavour, α reductase inhibitor, hypocholsteremic,), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 3-Hydroxy-2,3-dihydromaltol (antioxidant, anti-inflammatory and anti-cancer). The biological activities of phytocompounds are with reference to Dukes phytochemicals book [20].

The compounds are mostly of fatty acids, terpenoids, flavonoids and other small functional groups. The presence of phytocompounds varies widely in each sample as the location of the sample also varies. The phytocompounds present in Tamarind pulp are of great biological importance because of the presence of various antioxidants. Appropriate amount of intake of tamarind pulp in our diet may provide long term health benefit. Thus, the GC-MS studies are further taken for *in silico* work with the compounds identified for anti-cancer activity.

In silico analysis (Docking studies)

The qualitative phytochemical analysis revealed the presence of potent phytocompounds. Further, GC-MS analysis confirmed the presence of fatty acids, terpenoids, flavonoids and other small functional groups. From the GC-MS analysis, the compounds were screened which were related to anti-cancer property. Among the compounds screened, Squalene, Gamma-sitosterol, 3-Hydroxy-2,3-dihydromaltol from sample-1, sample-2 and sample-3 was found to be related to the present study having anti cancer activity. Further these compounds were docked by molecular docking. Squalene, a triterpenoid which has an anti cancer property and also used as an immunologic adjuvant in vaccines [21]. Gamma-Sitosterol**,** a steroid which exerts a potential anti-cancer activity through growth inhibition, cell cycle and apoptosis in cancer cells [22]. 3-Hydroxy-2,3-dihydromaltol, a flavonoid which has an anti-inflammatory property. Leucovorin calcium was used as standard.

Using PatchDock software the bioactive compounds of Tamarind pulp were docked against the protein that showed the anti-cancer activity for colon cancer through docking scores. The docking analysis of squalene [figure:1], Gamma-sitosterol [figure:2], 3-Hydroxy-2,3-dihydromaltol [figure:3] from fresh Tamarind pulp of methanol extract showed docking scores (5266, 4318 and 3130) and atomic contact energy (-278.15, -108.91 and -104.42) against the target C-reactive protein. Among the three compounds docked against the target protein, the compound squalene showed higher docking score and negative atomic contact energy. Docking analysis of standard drug Leucovorin calcium showed docking score of 4668 and atomic contact energy (-316.73) [figure:4]. When the phytocompounds are compared with the standard drug, squalene showed higher binding score and atomic contact energy. This revealed that the phytocompound squalene from Tamarind pulp was in good fit with the target protein in 3D space than the other two compounds and standard drug which indicated the good binding efficacy and geometric complementarity score of the phytocompound- Squalene against the target protein which is used for the inhibition of colon cancer. *In silico* docking analysis is a virtual screening method which helps to assess the potency of promising phytocompounds for discovery of novel drugs from a huge compound library by predicting the similarity of 3D structure of compound with the known ligand based on binding score, geometric patch complementarity score, docking fit and negative atomic contact energy [23] The higher binding score and negative atomic energy between the ligand and target leads to the (*In vivo* studies with particular compound and the ligand) development of novel anti-cancer drug [24]. The elevated level of CRP increases the risk of cancer [25] which in turn reduces the life span of patients. It was suggested that Tamarind pulp is effective against the colon cancer [26]. Thus, docking results are the

clear evidence for Tamarind pulp having anti cancer properties. Especially Squalene which has an anti tumour activity helps to inhibit the formation of lesions [27] caused by the C-reactive protein.

CONCLUSION

The present study revealed that the Tamarind pulps are enriched with phytoconstituents such as carbohydrates, terpenoids and flavonoids. The GC-MS analysis revealed the presence of bioactive compounds. Further docking studies confirmed the presence of Squalene, a triterpenoid showing higher binding affinity against the target protein CRP for colon cancer. Tamarind pulp which has been used in cooking mainly for its sour taste. In addition to this it has important phytocompounds with disease preventive and health promoting properties. Further in vivo studies is been carried out for isolation of specific phytocompounds.

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REFERENCES

1. World Cancer Research Fund and American Institute for Cancer Research Food, Nutrition, Physical Activity, and the Prevention of Cancer. A Global Perspective. Washington, DC: American Institute for Cancer Research, 2007.
2. World Health Organization Cancer Incidence in Five Continents. Lyon. The World Health Organization and The International Agency for Research on Cancer, 2002.
3. BoyleP, Langman J.S .ABC of colorectal cancer: Epidemiology. The bmj 2000; 321(7264):805-8.
4. National cancer Institute, All India Medical Sciences; cancer statistics. http://nciindia.aiims.edu/en/cancer-statistics. 2020.
5. Kuipers E.J, Rösch .T and Bretthauer .M. Colorectal cancer screening--optimizing current strategies and new directions. Nat Rev Clin Oncol., 2013; 10(3):130-42.
6. Ei- Siddig K, Gunasena H, Prasad B, Pushpakumara D, Ramana K, Vijayanand P, and Williams J. Tamarind, *Tamarindus indica* L. Fruits for the future 1. (revised edition) CUC- Southampton centre for underutilized crops, 2006.
7. Lewis, G. Schrire, B. Mackinder, B. and Lock, M. (eds.) .Legumes of the World. Royal Botnaic Gardens, Kew, 2005.
8. TNAU agritech horticulture, Tamarind (*Tamarindus indica* L.) Caesalpiniaceae. www.agritech.tnau.ac.in
9. Hiwale.S. Tamarind (*Tamarindus indica* L.). In: sustainable Horticulture in semiarid dry lands. Springer 2015; 443.
10. Tropical Plants Database, Ken Fern. tropicaltheferns.info. *Tamarindus indica* useful tropical plants.
11. Raaman N. Phytochemical Techniques. New India Publishing Agency 2006; pp. 19-25.
12. Hossain MA and Nagooru MR. Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Corydyline terminalis* L. Kunth. Pharmacogn 2011; 3:25-30.
13. Mamta Saxena, Jyoti Saxena, Rajeev Nema, Dharmendra Singh and Abhishek Gupta. Phytochemistry of medicinal plants. J Pharmacogn Phytochem 2013; 1:168-82.
14. Kokate KC. Practical pharmacognosy (4th edition) Vallabh Prakashan, Delhi, 1997; pp. 218.
15. Pinar kuru. *Tamarindus indica* and its health related effects. Asian pacific Journal of Tropical Biomedicines 2014; 4(9): 676-681.
16. Abiraami Valli.S and Uma Gowrie. S. A study on the bioactive potential of fresh and dried sprouts of *Cocos nucifera* L.-An invitro and in silico approach. International Journal of Pharmacy and pharmaceutical sciences 2017; 993:129-142.

17. McGarvey DJ and Croteau R. Terpenoid metabolism. Plant Cell 1997; 7:1015-26.
18. Indumathi, Durgadevi, Nithyavani and Gayathri. Estimation of terpenoid content and its antimicrobial property in *Enicostemma litorrale*. Int J ChemTech Res Coden (USA) 2014; 6:4264-7.
19. Syed Zameer Hussain and Khushnuma Maqbool. GC-MS: principle, technique and its application in food science. International Journal of Current Science 2014; 13:116-26.
20. Duke. J. A. Handbook of Biological Active Phytochemicals & Their Activity. 1st edition ,CRC Press, 1992.
21. Mala.R, Ruby Celsia.A.S, Malathi Devi.S and Geerthika.S. Comparison on bactericidal and cytotoxic effect of silver nanoparticles synthesized by different methods. IOP Conf.Ser. Mater. Sci. Eng. 2017; 225 012155.
22. Shenbagamoorthy Sundarraj, Ramar Thangam, Vellingiri Sreevani, Krishnasamy Kaveri, Palani Gunasekaran, Shanmugam Achiraman and Soundarapandian Kannan. γ-Sitosterol from Acacia nilotica L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells, J Ethanopharmacol 2012; 141[3):803-9.
23. Kowit Hengphasatporn, Arthur Garon, Peter Wolschann, Thierry Langer, Shigeta Yasuteru, Thao N.T. Huynh, Warinthorn Chavasiri, Thanaphon Saelee, Siwaporn Boonyasuppayakorn and Thanyada Rungrotmongkol. Multiple Virtual Screening Strategies for the Discovery of Novel Compounds Active against Dengue Virus: A Hit Identification Study. Scientia Pharmaceutica 2019; 88(2).
24. Hridya Hemachandran , Amrita Anantharaman , Sankari Mohan , Gopalakrishnan Mohan, Thirumal Kumar. D, Diksha Dey , Drishty Kumar , Priyanka Dey , Amrita Choudhury , George Priya Doss. C and Siva Ramamoorthy. Unraveling the inhibition mechanism of cyanidin-3-sophoroside on polyphenol oxidase and its effect on enzymatic browning of apples. Food Chemistry 2017; 102-110.
25. Kristine H Allin, Borge G Nordestgaard. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. Crit Rev Clin Lab Sci 2011; 48(4):155-70.
26. Flavia Martinello, Vinicius Kannen, Joao Jose Franco, Bianca Gasparotto, Juliana Yumi Sakita, Atushi Sugohara, Sergio Britto Garcia and Sergio Akira Uyemura. Chemopreventive effects of a *Tamarindus indica* fruit extract against colon carcinogenesis depends on the dietary cholesterol levels in hamsters.Food Chem Toxicol 2017; 107 [ptA]: 261-269.
27. Chinthalapally. V. Rao, Harold L. Newmark and Bandaru S.Reddy. Chemopreventive effect of Squalene on colon cancer. Carcinogenesis 1998; 19(2):287-90

Table 1: Qualitative phytochemical analysis of methanol and aqueous extracts of Tamarind pulp

S.No.	Phytochemical constituents	Methanol	Aqueous
1	Alkaloids	-	-
2	Glycosides	+	+
3	Saponins	+	+
4	Phenols	+	+
5	Terpenoids	+	+
6	Tannins	+	-
7	Flavonoids	+	+
8	Carbohydrates	+	+
9	Proteins	-	-
10	Aminoacids	-	-

*[+]=Present;[-]=absent.

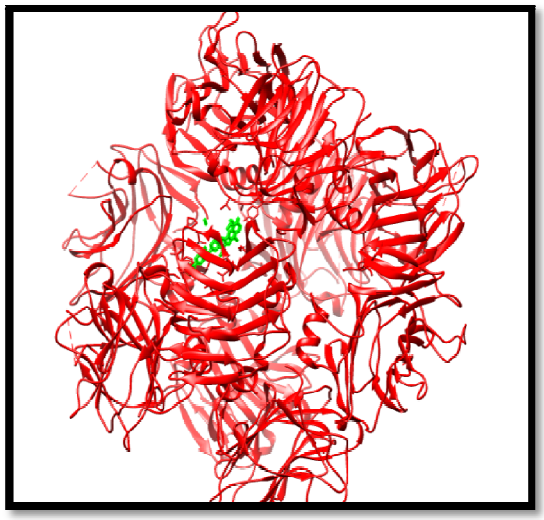

Figure:1 Docking of squalene with CRP

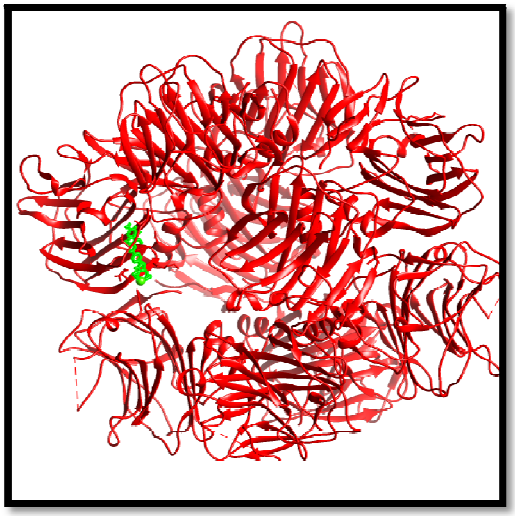

Figure:2 Docking of γ-sitosterol with CRP

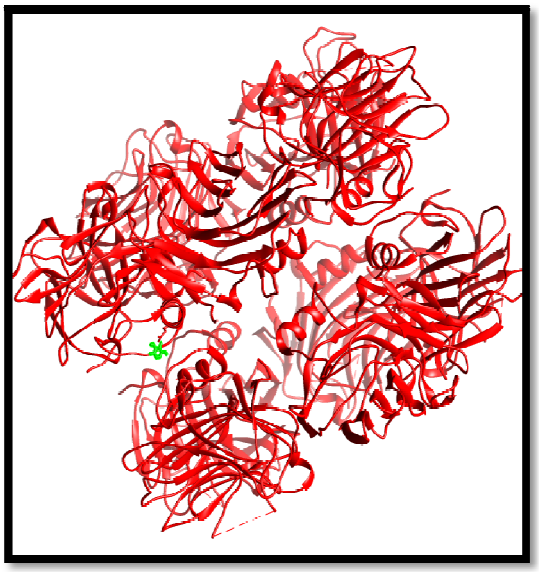

Figure: 3 Docking of 3-Hydroxy-2,3-dihydromaltol with CRP

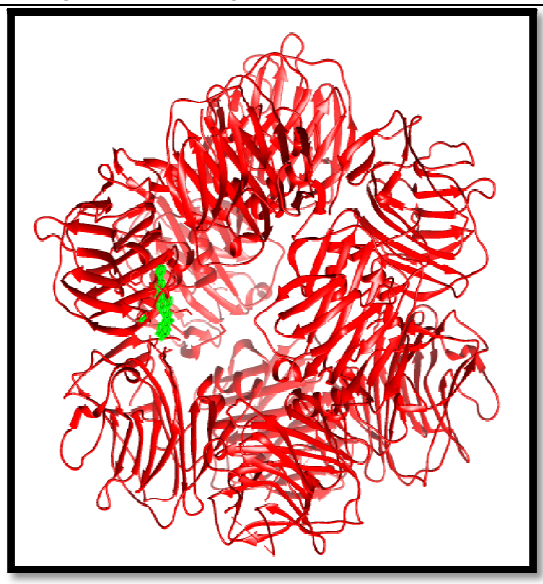

Figure: 4 Docking of Leucovorin calcium (Standard drug) with CRP

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RESEARCH ARTICLE

Phytochemical Screeing and GC-MS Studies and Antioxidant Activities of *Ruta chalepensis L.*

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ABSTRACT

Ruta chalepensis L. is a prominent source of essential secondary metabolites. Moreover, it is a medicinal plant and is still used in traditional medicines. The leaves of *Ruta* were screened for the presence of bioactive compounds. The dried aerial leaves of the plants are extracted by ethanol, methanol, and chloroform solvents. The phytochemical results of *Ruta chalepensis L.* show its richness in tannins, alkaloids, and phenols in all three extracts. Whereas saponins are rich in chloroform extract which is an active compound in antimicrobial activity. Most of the active compounds were extracted from the aqueous extract compounds namely terpenoids, carbohydrates, tannins, flavonoids, quinines, coumarins, and amino acids. The presence of variations in phytochemical groups can be used as promising support for the potential occurrence of biological activities. Quantitative analysis was performed to identify the bioactive compounds in the extracts by using GCMS. Sixteen compounds were characterized in the extract and were expressed as a % based on peak area and retention time. The result revealed the 5 bioactive compounds in the chloroform extract, 10 compounds in ethanol extract, and 1 compound in methanolic extract. These determined major compounds are Dodecane, Nonane, 2-undecanone, 2(5H)-Furanone, 3-methyl, 1-Octadecanesul phonyl chloride, and El Cosyl nonyl ether. The bioactive compounds characterized in the extract of *Ruta chalepensis L* have a prominent effect on various diseases.

Keywords: *Ruta chalepensis* L, Phytochemical screening, GCMS analysis, antioxidant activity

INTRODUCTION

Infectious diseases caused by microorganisms are a critical health challenge and they are supposed to be one of the chief causes of rising rates of morbidity and mortality worldwide. For numerous decades, natural remedies and medicinal plants were the major, and, only, a resource for physicians. *Ruta chalepensis* L normally called *Rue*, is an aromatic evergreen shrub, belonging to the family Rutaceae is a basis of the massive variety of natural products with

antibacterial, antifungal, antioxidant, actions (Raghav *et. al*. 2006). The consequence of plants to homeopathy and modern medicine is coexisting to their chemical constituents such as terpenoids, phenolics, alkaloids, flavonoids, amino acids, saponins, glycosides, diterpenes, triterpenes, and their compatibility with the human body (Fakhfakh *et. al.* 2012). *Ruta* is also one of the most often used plants for medicinal purposes. In several countries, it is cultivated for its pharmacological and biological activity and it is broadly used for the treatment of gastric, diuretic, inflammation, headache, and rheumatism disorders. Phytochemicals are compounds that have defensive or disease preventive properties naturally synthesized by plants. They are generally called plant secondary metabolites (Hayashi *et. al.* 2002)

Various extract of the aerial parts of *R. chalepensis* was studied for its antipyretic, anti-inflammatory, analgesic and CNS depressant activities (Merghache *et. al*. 2009). Natural products of these plants has antimicrobial agents with novel mechanisms of action. The present study was focussed to determine the quantitative analysis of phytochemical composition and the antioxidant activities of *Ruta chalepensis L.*

MATERIAL AND METHODS

Biological material

The aerial part (leaves) of *Ruta chalepensis L.* was collected on February 2021 around Ugarthenagar, Kodaikanal. Then botanical identification was carried out based on several morphological criteria and various books of botany and medicinal plants.

Sample preparation

The collected leaves were cleaned and dried at RT (25 °C) for 4 days under the shade and grounded to powder. Then packed and stored in an air-tight container. Finally, 250gm of leaf powder was obtained. Then the dried aerial parts of the plant were extracted by ethanol, methanol, and chloroform solvents (Szollosi *et al.,* 2002).

Moisture content

The water content present in the *Ruta chalepensis L.* was calculated by the oven drying method at 35 °C. 10g of the fresh leaves were placed in the oven at 35 °C for 24 h and repeated 3 times to obtain average moisture content (Liu *et al.,* 2001). The Moisture content (H%) is calculated by the following formula:

$$H\% = (mf - md) \div mf \times 100$$

Where:

H- Moisture content, Mf- Masses of the fresh plant. Md- Mass of the dry plant.

Phytochemical screening

Qualitative analysis

The plant powder of *Ruta chalepennsis L.* was used for preliminary phytochemical screening and determination of phytochemical groups according to the method described by (Ayoola *et. al.* 2008). The phytochemical tests were performed to detect alkaloids, saponins, terpenoids, carbohydrates, tannins, flavonoids, phenols, quinines, glycosides, coumarins, and amino acids according to the method described by Bruneton (2009).

Sample preparation and Extraction

The fine powder was soaked with different solvents ethanol, methanol, and chloroform for 3days. The filtrate was dried using a rotary evaporator at 40° C. Then screening was done according to the standard procedure. phytochemical screening of leaf extract of *Ruta chalpensis* L solvent extracts was carried out with the following procedures (Kumar *et. al.* 2009).

Test for Carbohydrates

2ml of the solvent extract was treated with 10 drops of Benedict's reagent and heated gently. The presence of carbohydrates was confirmed by orange-red precipitate.

Test for Tannins

1ml of the solvent extract was treated with 2ml of 5% ferric chloride. The appearance of dark blue or greenish-black color precipitate indicates the presence of tannins.

Test for Saponins

2 mL of each solvent extract were mixed separately with 10 mL of distilled water and then agitated (shaken) in a graduated cylinder vigorously for 15 minutes. The presence of saponins was confirmed by the formation of foam.

Tests for Alkaloids

Mayer's test

2 mL of each solvent extract were evaporated separately to dryness and the residue was heated on a boiling water bath with 2 mL of 2 N HCl. Then the mixtures were allowed to cool after 5 min 2 drops of Mayer's reagent were added. The presence of alkaloids was confirmed by the presence of turbidity.

Test for flavonoids

Alkaline test

2 mL of each solvent extract were treated separately with 0.5ml of 2N sodium hydroxide solution. The formation of intense yellow color indicates the presence of flavonoids.

Test for Glycosides

Salkowski's test: 2 mL of each solvent extract were mixed separately with 2 mL of chloroform. Then 1 mL of concentrated H_2SO_4 was added in each carefully and shaken gently. The presence of glycosides was confirmed by the color change of reddish-brown appearance.

Test for cardiac Glycosides

To 0.5ml of each solvent extract, 2 ml of glacial acetic acid and few drops of ferric chloride were added, finally, H_2SO_4 is added. The formation of a brown ring at the interface is due the presence of cardiac glycosides.

Test for quinones

2ml of each solvent extract and 2ml of Con H_2SO_4 was added. The presence of quinines was confirmed by the color change, which is red in the colour.

Test for Phenols

Ferric chloride test

2ml of each solvent extract was treated with 0.5 ml ferric chloride. The appearance of bluish color solution shows the presence of phenols.

Test for Terpenoids

To the 1 mL of each solvent extract, 1 mL of acetic anhydride and 1 mL of concentrated H_2SO_4 were added. The presence of terpenoids was confirmed by the formation of the bluish-green color ring on the tubes.

Test for Amino Acid

Ninhydrin Test

2ml of each solvent extract was treated with few drops of 0.2% ninhydrin reagent and heated for 5 min. The amino acids presence were confirmed by the appearance of blue color in the tubes

Test for Coumarins

To the 1 ml of each solvent extract, 1ml of 10% Sodium hydroxide was added. The presence of coumarins was confirmed by the appearance of yellow color.

Test for Anthraquinones

To the 1ml of each solvent extract, few drops of 10% ammonia solution were added. The presence of anthraquinones was confirmed by the presence of pink color precipitate.

Test for Steroids

To the 1ml of each solvent extract and an equal volume of chloroform was added. A few drops of concentrated H_2SO_4 have added the appearance of a brown ring indicates the presence of steroids and the appearance of the bluish brown ring indicates phytosteroids presence.

Test for Phlobatannins

To the 1ml of each solvent extract, few drops of 2% HCl were added. The appearance of red colour precipitate indicates the presence of phlobatannins.

Test for Anthracyanin

To the 1ml of each solvent extract, 1ml of 2N Sodium hydroxide were added and then heated for 5 min at 100 °C. The presence of anthracyanin was confirmed by the formation of the bluish-green color precipitate.

Quantitative analysis

GCMS Analysis

In Gas Chromatography (GC)- aqueous extract was injected into a port, they undergo heating up to 300 °C and the material is then volatilized. The column is wound within a unique oven which controls temperatures from -20° to 320°. The column surface is coated with a material that will separate the range of chemical compounds in the sample depend on the size and/or polarity. Sample components that are further volatile and smaller will travel through the column rapidly than others. Analysis in the Mass Spectrometer (MS)- The separated components flow straightly out of the column and undergo ionization whereas the components are blasted with electrons, causing them to break up and sprint into positively charged ions. In a filter, the ions flow through an electromagnetic field and filter through as they exceed the ionization sources. Then in the detector, counting of the number of filtered ions will occur, then the information is sent to a computer and a mass spectrum, ultimately sharing of ions of diverse size is generated. The identification of chemical constituents was based on the co-injection of authentic compounds on the similarity of the retention indices (RI) and by computerized matching on the acquired mass spectra with the stored in spectrometer database using the Nist mass spectral library and the literature (Adams *et al.,* 1995)

Antioxidant activity (DPPH (1-diphenyl-2-picrylhydrazyl) radical-scavenging assay)

The antioxidant activity of ethanol, methanol, and chloroform extracts of *Ruta chalepennsis* was determined using the DPPH free radical scavenging assay according to the method described by Yen and Duh (1994). Different concentrations (50-500 µg/ml) of the extracts were taken in different tubes. Fresh DPPH solutions 2 ml, 0.06%, w/v were prepared in methanol and added 1 ml in each tubes containing extracts. The reaction mixtures and the standard (ascorbic acid) were vortexed and stored at RT for 30 minutes and absorbance was measured at 520 nm. The percentage inhibition (PI) of the DPPH radical was measured with the formula(Yen and Duh, 1994):

$$PI = \frac{(AC - AS)}{AC} \times 100$$

where AC is Absorbance of the control and AS is Absorbance of the sample.

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemicals in *Ruta chalepensis L.* with methanol, ethanol, and chloroform were listed in Table 1. The qualitative analysis of ethanolic extract showed the presence of more phytochemicals than chloroform and methanolic extracts. The results revealed that the phytochemical screening of *Ruta chalepensis L*. indicated the richness in tannins, alkaloids, and phenols in all three extracts. Whereas saponins are rich in chloroform extract which is an active compound in antimicrobial activity. Most of the active compounds were extracted from the aqueous extract compounds namely terpenoids, carbohydrates, tannins, flavonoids, quinines, coumarins, and amino acids. *Rue* extracts were already reported to scavenge hydroxyl radicals and inhibit lipid peroxidise at low concentration (Mahmoudi *et. al.* 2016). The analysis showed the presence of phenolic compounds which have tremendous antimicrobial potential. The aqueous extract showed the presence of terpenoids which mainly involves in the reduction of sugar in the blood and are extensively used in disinfection and cellular defense mechanism in atherogenesis and cancer.

Analysis of GCMS profile

The GCMS analysis was used to evaluate several bioactive compounds used in medical fields, cosmetics, and food industry. In the present study characterization of the chemical profile of *Rue* using GCMS was done. In fig 1gas chromatogram showing the relative concentration of various compounds eluted as the function of retention time in the chloroform extract. The height of the peak represents the relative concentrations of the compounds in the leaf extracts. Mass spectrometer analysis of the compounds eluted at different times to identify the structure of the bioactive compounds. The larger compounds fragment to the smaller compounds giving rise to the appearance of the peak at different m/z ratios. These mass spectra are finger print of the exact compounds which are recognized from the library. The presence of alkaloids, ketone, phenolic compounds and flavanoids was observed this phytocompounds plays key role in pharmaceutical industries. Alkaloids are competent in pharmacological activities like analgesics and anti-malarial. Flavonoids are prominent for anti-oxidant, anticancer, and anti-inflammation property.

GC analysis was coupled with mass spectrometry to identify the volatile compounds produced by *R. Chalepensis*. Table 2 represents the complex chemicals composition present in the extracts. The chloroform extracts showed 5 bioactive compounds such as 2-Undecanone (12.55%),6.10-dimethyl, 2-Undecanone (28.88%), Carbonic acid,nonyl vinyl ester (21.29%), 1-Octadecanesul phenyl chloride (26.46%), Dodecane (10.81%). The ethanolic extract showed the presences of 10 bioactive compounds namely 2(5H)-Furanone,3-methyl (8.11%), (R)-(+)-3-Methyl cyclopentanone (28.98%),2-Butenedi amide,(Z) (1.82%), Nonane (3.76%), Benzene,1,3-dichloro (2.76%), 2-Ethyl-1-hexanol (19.6%), Cyclopropane,1,1-dichloro-2-met (5.2%), Dodecane (11.97%), 2-undecanone (4.76%), Tetradecane (13.68%). The methanolic extract showed the presence of 1 bioactive compound namely El Cosyl nonyl ether (100%)

Due to the presence of different bioactive compounds in the extract of *Rue*, it has potential applications in various pharmaceutical industries (Hadis *et. al.* 2003). Results confirmed the presence of numerous flavonoids, ketone and phenolic compounds in the leaf of *Rue* extract. The active compounds present in the *Rue* extracts, which imparts its medical value.

Biological evaluations

Antioxidant activity by DPPH

Total antioxidant activity of *Ruta chalepensis L.* crude extract was calculated based on the radical scavenging effect of the stable 1,1-dephenyl-2 picryhydrazyl (DPPH)-free radical activity to form the corresponding hydrazine. The degree of a streak of the violet color of DPPH radical, as it gets compact, indicates the radical scavenging potential of the antioxidant. The methanol extract of R. chalepennsis showed maximum scavenging activity at 96%, and ethanol extract at 95%, and chloroform extract at 95% this indicates the high radical effect. The obtained results of the antioxidant activity indicated that all the extracts showed higher antioxidant activity. Due to the presence of a wide range of phenolic substances extracts show strong antioxidant activities

CONCLUSION

In this study phytochemical screening of *Ruta chalepensis L.* exhibited the presence of different chemical groups. Most of the active compounds were extracted from the aqueous extract. The phytochemical results of *Ruta chalepensis L.* show its richness in tannins, alkaloids, and phenols in methanol, ethanol and chloroform of all three extracts. Saponins are rich in chloroform extract which is an active compound in antimicrobial activity. In addition, these compounds are synthesized through secondary metabolism and their construction and accumulation might diverge according to the species and the environmental conditions. The degree of discoloration of the blue color of DPPH radicals, as it is concentrated and indicated the radical scavenging potential of the antioxidant. All the extracts showed maximum antioxidant activity. Further studies are required to recognize the biologically active compounds and to assess the efficiency of the compound against pathogenic microorganisms associated with different human diseases.

REFERENCES

1. Adams. R; 1995. Identification of essential oil components by gas chromatography/mass spectroscopy, Allured Publ. Corp., Carol Stream, IL.
2. Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello Obaweya, A.A., Ezennia, E.C., Atangbayila, T.O., 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. Trop. J. Pharm. Res. 7 (3), 1019–1024
3. Bruneton 2009, Pharmacognosy, phytochrome and plant material, 4th ed.
4. Fakhfakh N, Zouari S, Zouari M, et al (2012) Chemical composition of volatile compounds and antioxidant activities of essential oil, aqueous and ethanol extracts of wild Tunisian Ruta chalepensis L. (Rutaceae). J Med Plants Res 6:593–600.
5. Hadis, M.; Lulu, M.; Mekonnen, Y.; Asfaw, T. Field trials on the repellent activity of four plant products against mainly Mansonia population in western Ethiopia. Phytother. Res. 2003, 17, 202–205.
6. Hayashi T; Maruyama H; Hatton K; Hazeki O; Yamasaki K; Tanaka T, (2002) Ellagitannins from Lagerstroemia speciosa as Activators of Glucose Transport in Fat Cells. Planta Med. 68(2), 173-175.
7. Kacem, M., Kacem, I., Simon, G., Ben Mansour, A., Chaabouni, S., Elfeki, A., Bouaziz, 2015. Phytochemicals and biological activities of Ruta chalepensis L. growing in Tunisia. Food Biosci. https://doi.org/10.1016/j.fbio.2015.08.001.
8. Kumar, A. Ilavarasn, R. Jayachandran, T. Decaraman, M. Aravindhan, P. Padmanaban, N. and Krishnan, M.R.V. (2009). Phytochemical investigation on a tropical plant. Pak. J. Nutri., 8, 83-85.
9. Liu F; Kim J; Li Y; Liu X; Li J; Chen X, (2001). An Extract of Lagerstroemia speciosa L. Has Insulin-Like Glucose Uptake–Stimulatory and Adipocyte Differentiation–Inhibitory Activities in 3T3-L1 cells. J Nutr. 131(9), 2242-2247.

10. Mahmoudi S, Khali M, Benkhaled A, Benamirouche K, Baiti I. Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian *Ficus carica L*. varieties. Asian Pac J Trop Biomed 2016;6:239-45.
11. Merghache S; Hamza M, Tabti B (2009) Étude physicochimique de l'huile essentielle de Ruta chalepensis L. de Tlemcen, Algérie. Afr Sci 5:67–81.
12. Raghav, S.K., Gupta, B., Agrawal, C., Goswami, K., Das, H.R., 2006. Anti-inflammatory effect of Ruta graveolens L. in murine macrophage cells. J. Ethnopharm. 104, 234–239.
13. Szollosi R, and Szollosi Varga I. (2002). Total antioxidant power in some species of Labiatae (Adaptation of FRAP method). Acta Biol Szeged. 46:125-127.
14. Yen, G.C., Duh, P.D., 1994. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen species. J. Agric. Food Chem. 42, 629–632.

Table 1 Qualitative analysis of phytochemicals in *R. chalpensis* with various solvents

S. No	Phytochemical constituents	Aqueous	Methanol	Ethanol	Chloroform
1	Carbohydrates	+	-	-	-
2	Tannins	+	+	+	+
3	Saponins	-	-	-	+
4	Alkaloids	-	+	+	+
5	Flavonoids	+	-	-	-
6	Glycosides	-	-	-	-
7	Quinones	+	-	-	-
8	Phenols	-	+	+	+
9	Terpenoids	+	-	-	-
10	Cardiac glycosides	+	-	-	-
11	Amino Acid	+	-	-	-
12	Coumarins	+	-	-	-
13	Anthraquinones	-	-	-	-
14	Steroids	-	-	-	-
15	Phlobatannins	-	-	-	-
16	Anthracyanin	-	-	-	-

(+ Presence, - Absence)

Table 2 Phytocomponents identified in the extracts of *R. chalepensis* by GC-MS.

List of compounds		
Chloroform	**Ethanol**	**Methanol**
2-Undecanone,6.10-dimethyl	2(5H)-Furanone,3-methyl	El Cosyl nonyl ether
2-Undecanone	(R)-(+)-3-Methyl cyclopentanone	
Carbonic acid, nonyl vinyl ester	2-Butenedi amide,(Z)-	
1-Octadecanesul phonyl chloride	Nonane	
Dodecane	Benzene,1,3-dichloro	
	2-Ethyl-1-hexanol	
	Cyclopropane,1,1-dichloro-2-met	
	Dodecane	
	2-undecanone	
	Tetradecane	

Table 3. The scavenging activity of DPPH radicals of ethanol, methanol, & chloroform extracts of *Ruta chalepennsis* L

Concentrations (mg/ml)	DPPH scavenging activity (%)		
	Ethanol	Methanol	Chloroform
50	35.678±1.5	42.25±3.6	45.10±1.3
100	42.52±0.9	46.12±1.5	50.02±0.6
200	50.65±1.6	53.72±0.2	54.14±1.2
300	76.53±0.8	76.86±3.5	65.25±0.8
400	85.12±0.7	87.14±1.3	78.14±1.8
500	95.2±02	96.3±2.4	95.57±2.3

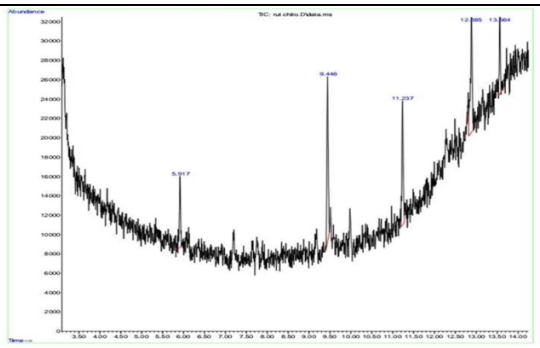

Fig. 1 Chromatogram of *Ruta chalpensis* L in chloroform extract

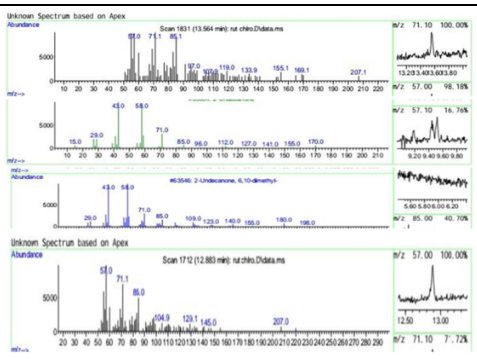

Fig. 2 Chromatogram of *Ruta chalpensis* L in chloroform extract

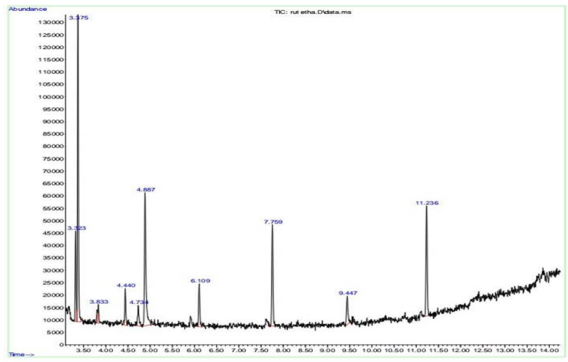

Fig. 3 Chromatogram of *Ruta chalpensis* L in ethanol extract

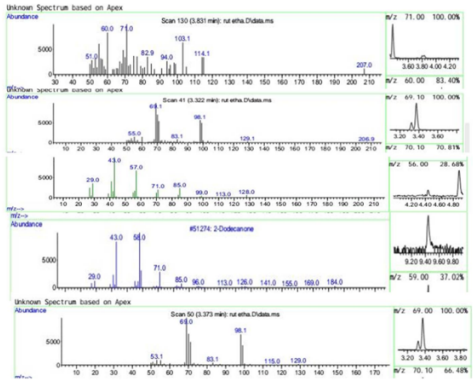

Fig. 4 Chromatogram of *Ruta chalpensis* L in ethanol extract

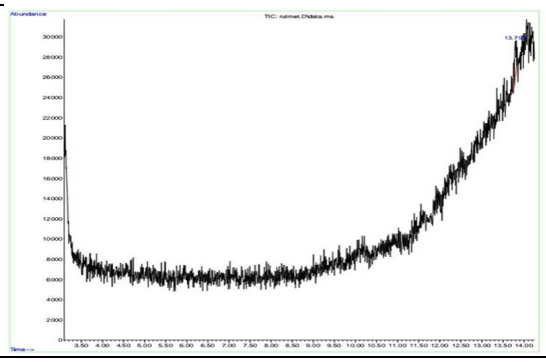

Fig. 5 Chromatogram of *Ruta chalpensis* L in methanol extract

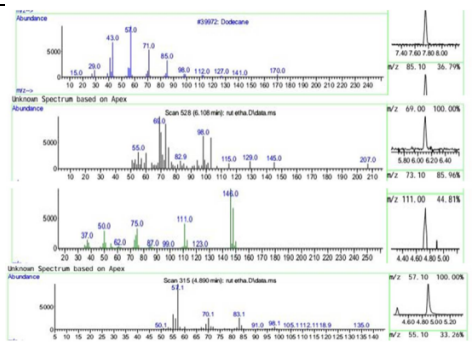

Fig. 6 Chromatogram of *Ruta chalpensis* L in methanol extract

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RESEARCH ARTICLE

The Role of Cooperatives in Enhancing Women Empowerment in Tamilnadu

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ABSTRACT

Empowerment is a multi-faceted, multi-dimensional and multi-layered concept. According to the Country Report of Government of India, "Empowerment means moving from a position of enforced powerlessness to one of power". This movement to a power position is not limited to men. Women empowerment is one of the major issues in the process of development of countries all over the nation. The state of Tamil Nadu has a glorious tradition of recognizing the importance of empowering women over several centuries now. Cooperatives has been used as a breeding ground for socio-economic empowerment of women. There are numerous women-based cooperatives in Tamilnadu that demonstrates the capability of women. Women possesses a great potential of developing her own business and improving her methodological skills and organizational self-help capacities (McKay, 2001). Cooperatives can create a safe environment where women increase their self-confidence, identify their own challenges, make decisions and manage risks. As a consequence, women are empowered and become dynamic agents of change, entrepreneurs and promoters of social transformation, thus improving their own lives the overall community on the whole. Cooperatives have been fruitful in not only enhancing social participation of women but also in developing drives, initiatives and leadership qualities. This conceptual paper tries to focus upon the vital role played by the cooperatives in enhancing women empowerment.

Keywords: Cooperatives, Enhancement, Women Empowerment

INTRODUCTION

It is estimated that there are some 7,50,000 cooperatives around the globe. They are providing memberships to a large number of people. Cooperatives provide a wide range of benefits, including affordable products and services, and ownership or control of resources (NCBA 2005; Ortmann and King 2007). There are several cooperatives in developing countries which focuses upon agriculture, with input supply and marketing as a key area of contribution (Holloway et al. 2000; Adeyemo 2004; Piesse et al. 2005; Ortmann and King 2007). Cooperatives have the ability to make a harmless atmosphere where women increase their self-confidence, identify their own challenges, make decisions and manage risks. As a consequence, women are empowered and become dynamic agents of change, entrepreneurs and promoters of social transformation, thus improving their own lives the overall community on the whole. Studies acknowledge that cooperative societies have a positive influence on various social aspects within communities, which often lead to social and economic benefits(Mayoux 1995; Hoyt 2004; Nyoro and Ngugi 2007). Studies that explore the role of cooperatives in enhancing women empowerment seems to be limited, but however a few of them do exist (see Oberhauser and Pratt 2004).

Cooperatives - Meaning

A cooperative is an independent association of people, who have come together voluntarily to meet out their mutual needs, such as, economic, social, and cultural needs and ambitions. They are a jointly-owned enterprise. Cooperatives are legitimately owned by their members. Each member has a right of one vote in electing the board of directors of the cooperative society. Cooperative organizations basically work to provide self-help and mutual help to individuals participating therein as members and also to the common people residing in that particular region.

Objective of a Cooperative Society

- The primary objective of any cooperative organization is to provide service to its members.
- They aim to provide goods and services.
- They aim to eliminate the unnecessary profits of middlemen in trade and commerce.
- They seek to prevent the exploitation of the weaker members of society.
- They aim to protect the rights of people both as producers and consumers.

Why Women needs Empowerment?

In our society, women are still face prejudice on their gender. They are not at all given equal rights as men. Women are paid less at work compared to their male counterparts. They are, expected to cook, clean and perform all the households in every situation. Women are always expected to strictly follow their culture and also carry it over to the next generation as well. In the Cambodian society, women are determined by their parents or husband only. Their individuality does not exist at all. They are not allowed to work or study far away from their home, as it is believed that women are weaker than men and some husbands get jealous out of it. In an addition to it, some women in Cambodia are married in young age and are also forced to get married by their parents. Women's empowerment is really needed in such societies. It's so important for women self-esteem and also for societies. Women, are always considered as the weaker sex of our society. They are often not included in many discussions at home. They are made to suppress themselves. Our societal structure is such which tries to suppress a woman in all dimensions. This in turn makes them weak and shatters their self-confidence. Women's empowerment is a part to encourage women to feel strong by telling them that they can do everything that they want to do. Women empowerment to a great extent reduces domestic violence towards her. When a woman is empowered, she imbibes a confidence to a great extent which supports her to face the society in all odd situations. Women empowerment has to begin with women's active participation. Unless women throw off the shackles that ignore their talent, skill and spirit women through education and economic self-reliance, cannot be empowered. Unless they are empowered to take a

decisive part in the social, political and economic life of the country the very development of the country will be lop-sided. The need for women's empowerment is felt because of the status they have in society since the beginning. Women happen to be the grassroots for a prosperous society. Of late, women are achieving high-level job just as man do. Women are now in various great and dignified positions in the society. They serve as president, leader, chief and other high-level positions. Also, there are several women now, who are actively participating in the society, politically, in the education front and economically. Economic empowerment increases women's access to economic resources and opportunities. If women's access to productive resources were the same as men's, women's contribution could reduce the total number of hungry people by 12 to 17 percent in support of Millennium Development Goal 1 of eradicating extreme poverty and hunger (FAO, 2011).

Now it has become a necessity to redefine the status of women in the society. A change can be brought through the constitution and supportive legislations. Even though our Indian Constitution gives a woman an equal status as men, but our society somehow tries to drag her to the backseat. Women have the ability to judge for themselves and take right decisions. Women have been excluded from centers of power as a result of systematic conspiracy by patriarchal thought most common in India Khap Panchayat that has relegated women to an allotted and confined space. A re-orientation of our attitudes towards women has to be carefully guided for their real emancipation from the patriarchal domination. There have been several attempts to reserve seats for women in political bodies. This is no doubt a step in the right direction. However merely allowing for reservation of women in Panchayat and legislative bodies without empowering women individually falls short of actual emancipation. The Government of India has made women empowerment as one of the major principal objectives of the Ninth Five Year Plan (1997-2002) and had also declared 2001 as the year of 'Women's Empowerment'. These issues of gender equality are discussed in World Conferences, National and International Conferences, etc. The Indian Constitution has deliberated and guaranteed equality before law, universal adult franchise and equal prospects for both men and women as a fundamental right. There is an imperative recognition of gender partnership in all the matters of societal development. In order to give a fillip to empowerment of women and appropriate institutional mechanisms and interventions have been consciously built into the development design. There are several institutions for the development of women and children at both the Central and State levels. The National Commission for Women and also State Commission for Women in several States are formed which are considered as some of the vital developments for the betterment and prosperity of women in the society. Various programmes such as Rashtriya MahilaKosh, Indira Mahila Yojana, Mahila Samridhi Yojana have been launched, reserving one third of the number of seats in Panchayats and the local bodies are programmes launched with a view to improve and empower women socially, economically and in political frontiers.

The Role of Cooperatives in Empowering Women

People of rural India face a lot of hardships to earn their livelihood. Most of them are engaged in some agriculture, animal husbandry and other subsidiary activities. Revenue from agricultural activities seem to be as erratic as the monsoon. Therefore, the people in the rural areas are forced to think of generating some additional revenues for their survival. Since these people are less literate and possess limited skills, the hunt for alternate source of income is constrained to a few occupations only. Women's empowerment has become a significant topic of discussion in the economic front. Her contributions, apart from home, if expanded to the society can bring great wonders. Here at this juncture, cooperatives come in to play a vital role in enhancing a woman's potential and empowering her. Cooperatives have a significant role to play as they are able to respond to both women's practical and strategic needs by providing access to income generating activities. There is a sense of recognition that women experience through cooperatives. They gain a reputed status, not only in their families, but also in the society.

Women empowerment is considered as a process in which women has an advantage of possessing a greater segment of control over resources - material, human and intellectual like knowledge, information, ideas and financial resources like money - and access to money and control over decision-making in the home, community, society and nation, and to gain 'power'. According to the Country Report of Government of India, "Empowerment means

moving from a position of enforced powerlessness to one of power". Cooperatives can also be supported to establish quotas for the participation of women in their leadership and to create women-only committees to ensure they can voice their concerns strongly enough to exercise leadership; conduct training activities to sensitize cooperative members to the negative impact of gender. A home driven by a woman is said to be pious as she infuses her imbibed values into it. Thus, if a cooperative is driven by women, the society on the whole becomes pious. Prakash, (2002) highlighted that social empowerment of a woman is a step-by-step process wherein they are allowed to exercise their rights and duties with self-confidence. Also, they are able to participate in the management process of their cooperatives. Many cooperatives like Aavin, employ women and provide a support in empowering themselves. They not only empower an individual woman but also stands as a proven example to the other women to come out and empower herself.

CONCLUSION

The cooperatives in the society have improved the lives of socially and economically backward women to a great extent. It has also increased their financial independence and security. Women's empowerment is possible through the development cooperatives in the society. Cooperatives has been identified as a source to address the financial needs of women. A cooperative society paves the way for women's empowerment in enhancing their socio-economic status, not only in rural areas but also in urban areas. Hence, cooperatives are important business model to work as a ground for reaping women empowerment mechanisms.

REFERENCES

1. Anbalagan, M., Amudha, R., & Selvam, V. (2005). Micro Credit to Self-Help Groups: A Boon for Economic Empowerment of Rural Women.
2. Desai V (2003), "Small scale Industries and Women Entrepreneurship", Himalaya Publishing House, Mumbai, pp.121-129,101-154
3. Easter, K. (1999). The gender gap in economic empowerment, Economic Analysis on employment and pay, India.
4. Jose, S., Chockalingam, D., & Velmurugan, D. (2019). Problems of Women Self Help Group Members in Ernakulam District. Journal of Critical Reviews, 7(1), 2020.
5. Karunakaran, R. (2004). An assessment of social impact of cooperative movement in Dindigul district. Unpublished PhD thesis. Gandhigram Rural University, India.
6. Madheswaran, (2001)" Empowering Rural women through SHG: Lessions from MRCP, Indian Journal of Economics, p.17
7. Nandhini, M., Usha, M., & Palanivelu, P. Women Empowerment through Self Help Groups: A Study in Coimbatore District
8. Pratheep, S., & Dharmaraj, A. (2016). An empirical study on stress levels among working women in export-oriented units of Tirupur district. Man in India, 96(9), 3079- 3087
9. Purna Chandra Parida and Aushree Sinha (2012), "Performance and sustainability of Self-Help Groups in India: A Gender persepective", P.56
10. S. Venkateshmurthy and G.M, Dinesh (2009) -Women Empowerment through SHG – An Analysis.
11. Subburaj, B. (2003). Economic contributions of cooperatives, Department of Cooperation, Gandhi gram Rural institute, Deemed University, Gandhi gram 624 302
12. Trivedi, Jigna; Priyan, P. K.; Bhinde, Vinay (2011), IUP Journal of Agricultural Economics, Vol. 8 Issue 1, p41-51. 11p
13. Venkatesh, J. & K. Kala. (2010). Empowering rural women all the way through self-help groups. International Journal of Management 1(2): 156-63.
14. Warman, M., & Kennedy, T. (1998). Understanding cooperatives: agricultural marketing cooperatives (USDA, Rural Business Cooperatives Service).

15. Young, K. (1992). Household resource management, gender and development: A practical guide. Ostergaard L. (ed.). Routledge London and New York
16. https://ncbaclusa.coop/resources/what-is-a-co-op/
17. https://ncdc.unl.edu/what-cooperative
18. https://timesofindia.indiatimes.com/readersblog/joonakkonwar/women-empowerment-3-26133/
19. https://www.azadindia.org/social-issues/need-for-women-empowerment.html
20. https://www.ica.coop/en/cooperatives/what-is-a-cooperative#:~:text=Cooperatives%20are%20people%2Dcentred%20enterprises,a%20democratic%20and%20equal%20way.
21. https://www.ilo.org/empent/units/cooperatives/lang--en/index.htm
22. https://www.isas.nus.edu.sg/papers/women-empowerment-in-india-more-needs-to-be-done/
23. https://www.unfpa.org/resources/issue-7-women-empowerment
24. https://www.worldvision.com.au/womens-empowerment/
25. https://www.worldvision.org/gender-equality-news-stories/seven-ways-empower-women-girls

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RESEARCH ARTICLE

Evaluation of *In vitro* Antiurolithiatic Activity of Jalotharimani using Struvite Crystal Growth Inhibition Assay

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ABSTRACT

Urolithiasis is the most common condition where urinary stones are formed anywhere in the urinary system. The incidence is quite high all over the world and it's the third most common disorder of urinary tract found in humans. It's estimated that at least 10% of world's population in industrial region is afflicted by urinary calculi. In Siddha system of medicine there are numerous formulations with herbal and mineral ingredients are used to prevent recurrence of urolithiasis. The present study was carried out to evaluate the in vitro Anti urolithiatic activity of Siddha Formulation *Jalotharimani* using struvite crystal growth inhibition assay. The results indicated that the test drug *Jalotharimani* has significant Anti urolithiatic activity in the tested medium and have clearly indicated that this formulation was quite promising for further studies in this regard.

Keywords: Anti urolithiatic activity,*Jalotharimani*, In Vitro, Siddha formulation

INTRODUCTION

Siddha system of medicine is the traditional healing system followed mainly by South Indian peoples and it is considered as the one of the oldest system of Indian medicine. *Siddha* system is based on [1] *Saiva Siddhantham* and the Tamil word *Siddha* is derived from its roots "*siddh*", which means perfection in life or "Heavenly Bless[1]."[1]*Siddha* is based on the concept of man as a part of the universe and therefore on the harmony that exists between the two. The treatments are based on the three body humours *Vatha, Pitha and Kapha.* When there is derangement in these three humours, it results in diseases. *Kalladaippu* is one among the diseases caused due to the derangement of *Vatha* humour. During this condition the patient has symptoms of abdominal pain, vomiting, burning micturition, hematuria and pain in the low back etc. *Kalladaippu* is compared to the modern term Urolithiasis. Urolithiasisis a pathological condition of genitourinary system where there is formation of calculi or stones within the urinary tract. It includes the formation of stones in the parenchyma of the kidney, ureters, bladder or urethra. There are many causes which tends to increase the risk of an individual to develop Urolithiasis which includes change in the lifestyle habits, ingestion of certain dietary elements and also certain medications etc.,

Etiology

The causes of Urolithiasis are heterogeneous and vary depending upon the composition of stones [2,3,4]. 85% of renal calculi comprises of Calcium oxalate and phosphate stones which develops due to conditions such as hereditary hypercalciuria, hypocitruria, renal tubular acidosis and hyperparathyroidism [5]. While struvitestones comprise of only 15%. Struvitecalculi are named as infection stones due to their association with urea splitting bacteria, which degrade urea into ammonium and carbon dioxide and thus promotes the development of large, branched renal calculus. The most important urease producing bacteria are Pseudomonas, Klebsiella, Proteus and Staphylococcus species [6].

Epidemiology

Urolithiasis is a common finding in the general population with a prevalence range between 4 - 20 % [7]. It is estimated that about 1 in 1000 adults were hospitalized every year in US [8]. Urolithiasis is more common in men and approximately 7% of females and 13 % of males suffer from this condition by the age of 70[(8)]. However struvite stones develop in females because they are more commonly infected with urinary tract infections.In modern medicine removal of this struvite stone is done by surgical procedure and conservative measures like acidification, citrate administration and urease inhibitors were given in case of persistent infection [9]. Traditionally Urolithiasis can be prevented with herbs and minerals. In Siddha system of medicine there are so many formulations with herbal and mineral ingredients are used to prevent recurrence of urolithiasis. One among this is *Jalotharimani*, herbo mineral formulation from *Sastric Siddha* text *"Theraiyar Karisal 300"* is commonly used by Siddha physicians in their clinical practice. Keeping in view of this, this study is aimed to evaluate the invitro Anti urolithiatic activity of *Jalotharimani* using struvite crystal growth inhibition assay.

MATERIALS AND METHODS

Vengaram, Chukku, Milagu, Thippili, padigaram, KadalNurai, Suraivithai, Indhuppu – all those drugs were finely powdered. Then the mixture is grinded with *Perungyam* and *Nervalam*. Then *ilaneer* (tender coconut water) is added to the above mixture and grinded for 9 hours and made into 200mg pills and dried under shade [10] (Table 1).

Methodology

An aqueous solution of 0.5M Ammonium dihydrogen phosphate was admixed with appropriate amount of sodium metasilicate solution of specific gravity 1.05 in using magnetic stirrer so that the pH value 7.0. pH probe meter was used to ensure the pH. The test tubes of 140 mm length and 25 mm diameter was taken and 10 mL of gel solution was added to it. After the gel formation, 5 mL of supernatant solutions of 0.5 and test drug of concentration 1% in

magnesium acetate of 1.0 M concentration was poured on the set gels in test tubes to determine the growth inhibition of Struvite crystals. About 5 ml of 1.0 M magnesium acetate without test drug were added as supernatant to test tubes which serves as crystal control group. To avoid microbial contaminations all the procedures were done in the aseptic medium in laminar flow hood. All the glassware and test tubes and were autoclaved at a temperature of about 120°C for 15 min. After pouring supernatant solution, the test tubes were closed with air tight cap. Room temperature was maintained for the experiment. The Study on growth of crystal is carried out for 5 consecutive days [11].

RESULTS AND DISCUSSION

In modern medicine prevention of the recurrence of infection stones (struvite stones) can be achieved by integrated therapy. Complete clearance of the stone is only done by surgical procedure and in case of persistent infection conservative measures such as urease inhibitors, acidification and citrate administration were followed. To avoid surgical intervention and to prevent the recurrence of struvite stones herbal drugs are more efficient. The only drawback in the development of standard drug in *Siddha* system is the multifactorial nature of struvite stone due to its different chemical composition. In vitro crystal studies were widely used to study the crystal nucleation growth and aggregation processes. Different forms of struvite stones like dendritic type, rectangular platelet type, prismatic type and needle type were grown in the gel. The single diffusion gel growth technique was adopted to evaluate anti-urolithiatic potential of study drug *Jalotharimani*. Test drug was prepared at two different concentrations of 0.5 and 1 % dispersed in 1.0 M Magnesium acetate solutions.

Figure 1 shows growth of Struvite crystals in control gel medium. About 5ml of 1.0M Magnesium acetate prepared without test drug were added as supenatent to control tube which serves as crystal controls group. Figure 2 and 3 indicates the struvite crystals growth in gel medium with 0.5 of 1% of *Jalotharimani* which is prepared by 5ml of supernatent solutions of 0.5 and 1 % concentration of test drug in 1.0M Magnesium acetate were gently poured on the set gels in test tubes to determine the growth inhibition of Struvite crystals. Then the growth of Crystal was carried out for five consecutive days. At the end of 5 days the size variation of Struvite crystals was observed. It is shown in the figure 4. 'A' indicates size variations of Struvite crystals in control group gel medium, 'B' indicates size variations of Struvite crystals in gel medium with 0.5 % of *Jalotharimani* and 'c' indicates size variations of Struvite crystals in gel medium with 1% of *Jalotharimani.*

The microscopic view of struvite stone was done. Figure 5 shows control gel medium, Figure 6 and 7 shows gel medium with 0.5% and 1%*Jalotharimani.* Table 2 represents the average length of the crystal in different medium. From the study it was observed that the average length of the crystal was higher in control medium (1.94), The average length of the crystal was reduced in 0.5% of test drug *Jalotharimani*(1.24) and its average length was very much reduced in 1% of test drug *Jalotharimani* (0.9).

CONCLUSION

The test drug *Jalotharimani* has significant Anti urolithiatic activity in the tested medium and have clearly indicated that this formulation was quite promising for further studies in this regard.

REFERENCES

1. https://vethathiri.edu.in/siddha-treatment/siddha-medicine/
2. Ahlmen J. Incidence of chronic renal insufficiency: a study of the incidence and pattern of renal insufficiency in adults during 1966-1971 in Gothenberg. *Acta Med Scand Suppl.* 1975; 582:3.

3. Haddad CT. Highlights on the history of medicine in the Middle East. *TurkiyeKlinikleri J Med Ethics.* 2002;10: 243-257.Google Scholar
4. Preminger GM, Assimos DG. Chapter 1: AUA guideline on management of staghorn calculi: Diagnosis and treatment recommendations. *J Urol.* 2005;173: 1991-2000.
5. Ansari MS, Gupta NP et al. Spectrum of stone composition, structural analysis of 1050 upper urinary tract calculi from northern India. *Int J Urol.* 2005;12(1):12-6.
6. Sean P. Stroup, Brain K. et al. Urinary infection and struvite stones, Urinary Tract Stone Disease, P 217 – 224.
7. Trinchieri A. Epidemiology of urolithiasis: an update. Clin Cases Miner Bone Metab. 2008; 5(2):101-106.
8. Porter RS, Kaplan JL. Merck Manual of Diagnosis and Therapy. 19th Edition. Merck Sharp &Dohme Corp. Whitehouse Station, N.J; 2011.
9. Cohen T. D, Preminger G.M, Struvite calculi, SeminNephrol. 1996 Sep 16 (5); 425 – 34. PMID: Review.
10. Siddha Formulary of India Part I, Govt of India, Ministry of Health and Family Welfare, Fist edition, 1992, Page no.87.
11. Chauhan, C.K., K.C. Joseph, B.B. Parekh and M.J. Joshi, 2008. Growth and characterization of struvite crystals Indian. J. Pure Appl. Phys., 46: 507-512

Table 1. Preparation of *Jalotharimani*

S.No	Name of the ingredient	Botanical Name/ Chemical Name	Quantity
1.	*Vengaram*	*Borax*	35 grams
2.	*Chukku*	*Zingiberofficinale*	35 grams
3.	*Milaku*	*Piper nigrum*	35 grams
4.	*Thippili*	*Piper longum*	35 grams
5.	*Padikaram*	*Alum*	35 grams
6.	*KadalNurai*		35 grams
7.	*Suraivithai*	*Lagenariasiceraria*	35 grams
8.	*Indhuppu*	*Rock salt*	35 grams
9.	*Perungayam*	*Ferula asafetida*	35 grams
10.	*Nervalam*	*Croton tiglium*	35 grams
11.	*Ilaneer*	*Tender Coconut water*	Sufficient quantity

Table 2. Average Length of the Crystal in various medium

S.No	Medium	Average Length of the Crystals
1	Control Gel medium	
	Mean	1.94
	Std. Deviation	0.11
	Std. Error	0.05
2	Gel medium + 0.5 *Jalotharimani*	
	Mean	1.24
	Std. Deviation	0.40
	Std. Error	0.18
3	Gel medium +1% *Jalotharimani*	
	Mean	0.9
	Std. Deviation	0.1
	Std. Error	0.04

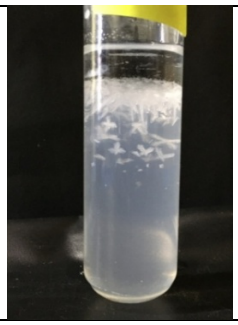


Fig. 1. Growth of Struvite crystals in control group test tube

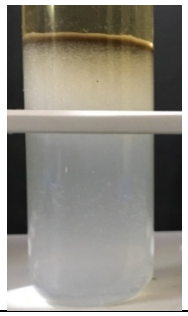


Fig. 2.Struvite crystals growth in Gel medium with *Jalotharimani* 0.5%

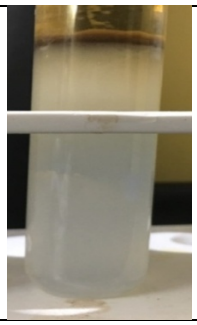
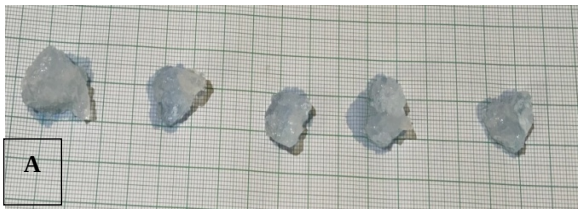


Fig. 3. Struvite crystals growth in Gel medium with *Jalotharimani*1%



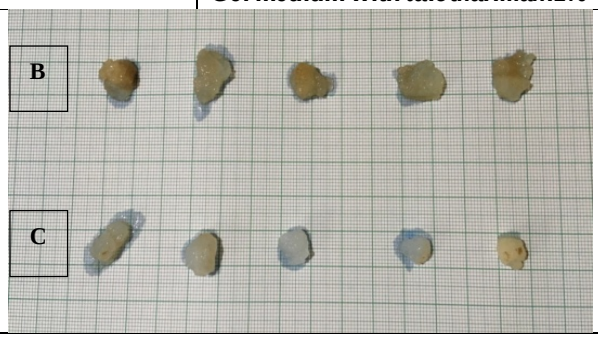


Fig.4 A - Size variation of Struvite crystals in Control Gel medium, B- Size variation of Struvite crystals in Gel medium with 0.5 % of *Jalotharimani,* C- Size variation of Struvite crystals in Gel medium with 1 % of *Jalotharimani*

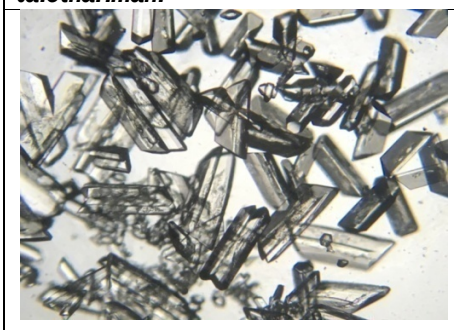


Fig. 5. Control Gel medium

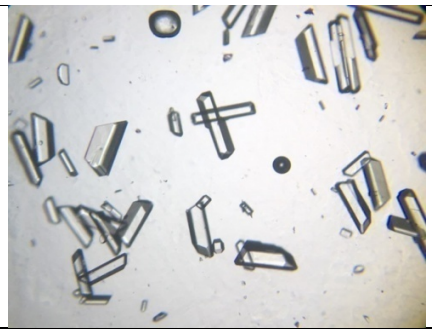


Fig. 6. Gel medium with 0.5 % of *Jalotharimani*

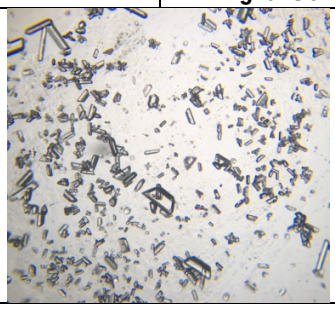


Fig. 6. Gel medium with 1 % of *Jalotharimani*

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RESEARCH ARTICLE

In silico Analysis of Violacein Producing Microbes and Evaluating the Efficiency of Violacein against Polio and Corona Virus

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ABSTRACT

Violacein, a secondary metabolite secreted by *Chromobacterium violaceum* is a valuable bisindole compound having antibacterial, antitumor, antimalarial, antiviral property and employed in dyeing industry for its purple color. The pigment is produced by various species such as *Janthinobacterium sp., Chromobacterium sp., Pseudoalteromonas sp.* Violaceum biosynthetic pathway requires an important gene cluster of *vioABCDE*. Yet, the *vioABCDE* genes have been studied only in *Chromobacterium violaceum.* Hence, this study was initiated to perform sequence and structural analysis of the Violacein genes and proteins among the *Pseudoalteromonas sps., Chromobacterium sps., Iodobacter fluviatilis , Janthinobacterium sps., Massilia sps., Myxococcus stipitatus sps.* and *Rivularia sps.* This study helped to trace the evolutionary origin of the violacein genes .Bioinformatic databases such as Genbank, KEGG and PubChem were used to identify the gene clusters and proceed with data analysis. Our results revealed that the violacein gene cluster was conserved with few sequence variations and structural changes. Phylogenetic analyses of the *vioABCDE* sequences revealed evolutionary lineage of the various genes among the organisms employed in this study. Interestingly, phylogenetic analysis revealed that a Violacein producing *Janthinobacterium sp B9_8* was highly similar with Violacein producing *Iodobacter species* than to other strains of *Janthinobacterium.* Protein structures of VioABCDE were also predicted to unravel the sequence-structure-function relationship and understand the mechanism of evolution. Structural studies revealed minor structural changes which did not affect the structure-function relationship. The violacein compound was docked for antiviral property using Polio viral capsid proteins VP1, VP2, VP3 and Corona virus spike protein. The *insilico* results revealed the violacein drug-target interaction with Polio virus Capsid protein but failed to bind at the active site of Corona virus spike protein. Additional Corona Viral proteins need to be employed for screening the antiviral nature of the violacein compound.

In addition, the diverse lineage of *Janthinobacterium sp B9_8* need to be traced to understand and trace its evolution.

Keywords: Violacein, Bacterial pigment, *insilico* gene comparison, *insilico* protein comparison, Docking study.

INTRODUCTION

The textile industry is responsible for a large list of environmental issues, one such is the use of chemical dyes. They are toxic, mutagenic and carcinogenic agents, occur as the environmental pollutants and cross entire food chains providing biomagnification, such that organisms at higher trophic levels show higher levels of contamination compared to their prey (Lellis *et al*., 2019). Keeping the effects of organic pollution in mind industries are finding an ecofriendly way of pigment production. Bacteria have some clear advantages as color resources compared to fungi. In the recent years, coloring of food with pigments produced from natural sources is of worldwide interest and is gaining importance. These pigments are looked upon for their safe use as a natural food dye in replacement of synthetic ones because of undesirable market (Tokkas *et al*., 2018). Pigments isolated from nature are believed to be free from carcinogens, toxins and are also biodegradable. Owing to the increased requirement for purple dye, violacein pigment is considered to have high commercial value in dyeing industry (Ratnakaran *et al*., 2020). Violacein is a violet bacterial purple pigment. Violet color as a dye has a significance from ancient times. Violet belongs to the family of blue color. Before the 1860s, the color violet was very uncommon.

Chromobacterium violaceum is the bacterium mainly focused on the violacein production studies. However compound violacein is also produced by many other bacterium species like *Janthinobacterium, Chromobacterium, Iodobacter, Collimonas, Microbulbifer, Duganella, Pseudoalteromonas* (Choi *et al*., 2015). Violaceins (including violacein and deoxyviolacein), a bis-indole structure containing blue-violet is produced by Gram-negative bacteria from different terrestrial and marine environments. Recently, violaceins were reported as metabolites for interbacterial competition in the *in vivo* level (Batista *et al*., 2020). Violaceins also display a number of health promoting activities, such as antibacterial (Cazoto *et al*., 2011), antioxidant (Konzen *et al*., 2006), antimalarial (Lopes *et al*., 2009) antitumor (Ferreira *et al*., 2004).

The biosynthetic pathway of violaceins was encoded in a single small operon *vioABCDE*. It starts from L-tryptophan via the shikimate pathway). Firstly, the (VioA) catalyses the conversion of L-tryptophan to indole-3-pyruvic acid (IPA) imine. Heme-dependent oxidase, VioB catalyses IPA imine monomer to dimer. IPA imine dimer is easily converted to chromopyrrolic acid spontaneously. Due to the instability of chromopyrrolic acid, it has been demonstrated that IPA imine dimer can be competitively transformed to protodeoxyviolaceinic acid via a specific non-cofactor containing enzyme (VioE) (Hirano *et al*., 2008). The two sequential reactions catalysed by VioB and VioE have been indicated as the rate-limiting steps in violaceins biosynthesis pathway (Balibar *et al*., 2006 , Zhou *et al*., 2018).

Chromobacterium violaceum is the bacterium mainly focused on the violacein production studies. In this compound the amino acid Tryptophan gets shunted into many microbial secondary pathways like violacein, rebeccamycin, and staurosporine which is formed from the oxidative dimerization of two Tryptophan (Tager *et al.,* 2018). Among this natural bisindole compound, violacein is a purple pigment.

The main objectives of this study are as follows:

Comparative sequence and structure analysis of violacein genes were carried out to identify the conserved and variant regions.

Identification of the conserved domain of the Violacein gene coded proteins and relating it to structure-function relationship. *In silico* - based evaluation of the antiviral activity of violacein against Polio viral protein and Corona Viral proteins.

METHODOLOGY

ENTREZ GENOMES

The nucleotide and the amino acid sequence of the selected organisms were retrieved in the fasta format and was aligned properly.

MULTIPLE SEQUENCE ALIGNMENT USING CLUSTAL OMEGA

Clustal Omega package was employed for constructing multiple sequence alignments of amino acid or nucleotide sequences, quickly and accurately.

Tree Top – Phylogenetic Tree Prediction

Treetop was used to construct the phylogenetic tree for Violacein gene sequences used in this study. The sequence alignment files were uploaded in this tool and bootstrapping option was selected for phylogenetic tree construction (Brodsky *et al*., 1995).

BLAST 2

It was used to compare the similarity and dissimilarity between the closely related and distant sequences. It produced the identity score, variation and gaps between two sequences in order to identify sequence variants,

INTERPRO

In this study INTERPRO database was used to identify the family and domain of the sequences used in this study (Blum *et al*., 2021).

Structure prediction

Secondary and tertiary structure prediction: NPS@ database was used to predict the secondary structures like helix, turns and loops of the protein sequences. SWISSMODEL tool was used to analyse the likeness and quality of three-dimensional structure of each protein sequences studied.

Compound analysis

The compound of interest was fully analysed using the Pubchem database. The Id of the compound from the server was used in docking study (Kim *et al*., 2019). The database provided information about the literatures, assays and property. SWISS ADME, ProTox ADME, pkCSM was used in predicting the drug efficiency and toxicity of the compound of interest.

Docking study

The PDB database was used to retrieve the viral structures in the PDB format for the docking analysis.
Supercomputing Facility For Bioinformatics and Computational Biology, IIT Delhi server was used for the active site prediction of the viral structures. AUTODOCK VINA was used for performing docking simulations and virtual screening tasks (Di Muzio *et al*., 2017). Pymol was used to visualize the binding efficiency of the drug to the compound for its efficiency in 3D format.

RESULTS AND DISCUSSION

***vioabcde* Genes and their Proteins**

Based on the information retrieved from the databases, protein sequence comparison will typically double the evolutionary lookback time compared to DNA sequences (Koonin *et al*., 2003). Due to these advantages, database sequence comparisons are typically carried out at *the* level of protein sequences in this study. *vioA* genes codes for tryptophan monooxygenase protein. The tryptophan monooxygenase has conserved domain of Flavin containing amine oxidoreductase family. The main role of amine oxidases is to provide a source of ammonium (Tavladoraki *et al*.,1998). *vioB* codes for iminophenyl-pyruvate dimer synthase VioB (Mulder *et al*., 2007). *vioC* codes for monooxygenases. The protein belongs to FAD binding domain of monooxygenases In this reaction, the two atoms of dioxygen are reduced to one hydroxyl group and one H_2O molecule by the concomitant oxidation of NAD(P)H (Schreuder *et* al., 1992)**.** *vioD* codes for Violacein biosynthetic enzyme E and is conserved in *Rivularia species*. The protein produced by the reference organism is Protodeoxyviolaceinate monooxygenase, while the other organisms it is expressed as tryptophan hydroxylase with 90% similarity according to UNIPROT. *vioE* codes for Violacein biosynthetic enzyme E, the protein belongs to VioE domain which plays a key role in Violacein synthesis.

Conserved Domain and Phylogenetic analysis

The study on conserved domain analysis of VioA, B and C are shown in Figure:1 A. The phylogenetic tree is highly reliable as they are constructed with bootstrapping value. The higher bootstrapping value (100) indicated higher reliability of phylogenetic relationship. The phylogenetic tree of *vioB* genes shows that *Janthinobacterium sp B9_8* to be clustered with *Iodobacter fluviatilis*. *vioC* genes shows *Myxococcus stipistatus* as the out group in the clustering. The phylogenetic analysis of VioC protein also confirmed *B9_8* is distantly related to the *Janthinobacterium species* and closely related to *Iodobacteria fluviatilis.* *Janthinobacterium B9_8* as a separate cluster from the *Janthinobacterium lividium* as shown in **Figure:2 AB.**

BLAST2 analysis

Blast2 analysis revealed that VioA protein showed 81% identity for *Janthinobacterium B9_8* and *Iodobacter fluviatilis* while the sequence comparsion between *Janthinobacterium sp B9_8* and *Janthinobacterium lividium* exhibited an identity of 41%. In VioB *Iodobacter fluviatilis* and *Janthinobacterium sp B9_8* sequence showed 84% identity, *Janthinobacterium sp B9_8* and *Janthinobacterium lividium* exhibited an identity of 55%. *Iodobacter fluviatilis* and *Janthinobacterium sp B9_8* of VioC showed 89% identity, while the BLAST2 result of *Janthinobacterium sp B9_8* and *Janthinobacterium lividium* revealed an identity of 63%. Similarity between *Iodobacter fluvitilis* and Figure 1, A shows the Conserved Domain Analysis of Vio ABCDE amino acid sequence in Q9S3V1- *Chromobacterium violaceum*, A0A5C1OJ31- *Chromobacterium sp 257-1*, A0A344UFD2-*Chromobacterium sp 11BBL-274-1*, A1X1N7- *Janthinobacterium lividium*, W0V275-*Janthinobacterium agaricidamnosm*, A0A0KIL9-*Massilis sp NR4-1*, A0A109RW37- *Janthinobacterium B9_8*, A1X1N7- *Janthinobacterium lividium*, A0A166XVA1- *Pseudoalteomonas luteoviolacea*, , K9RJ14-*Rivularia PCC 7116*, A0AIS6F854-*Janthinobacterium sp LM6*, A0A4Y6CGC3-*Myxococcus xanthus.* A: Conserved domain analysis of VioA

Phylogram analysis

Amino acid sequence phylogenetic analysis of VioB, VioC, containing organism in 2, AB

Phylogenetic analysis of VioB; B:Phylogenetic analysis of VioC

RAST analysis

Showing the conserved *vioA, vioB, vioC,* gene organisation similar to *Chromobacterium violaceum.*

A: RAST analysis of *vioA*; B: RAST analysis of *vio B*; C: RAST analysis of *vioC,* Figure: 4 A,B Structure of VioA in *Janthinobacterium lividium* and Ramachandran plot; C structure of Vio B showing poor quality; Figure: 5 Docking studies

Janthinobacterium sp B9_8 of VioC showed 89% identity, while the BLAST2 result of *Janthinobacterium sp B9_8* and *Janthinobacterium lividium* revealed an identity of 63%. Similarity between *Iodobacter fluvitilis* and *Janthinobacterium B9_8* for VioE revelaed 88% identity and the similarity between *Janthinobacterium B9_8* and *Janthinobacterium lividium* exhibited 51% identity.

RAST analysis

Though *Janthinobacterium sp B9_8* was distant from the other *Janthinobacterium species* and appears to be the distant group. RAST (Rapid Annotations using Subsystems Technology) analysis of *Janthinobacterium sp B9_8* showed that all the *vioabcde* gene organization is conserved and similar to the reference organism *Chromobacterium violaceum* as seen in Figure: 3 ABC.

Structural analysis

Earlier structural analysis was limited to the reference organism, *Chromobacterium violaceum* (Boggiano *et al*., 1958) Comparatively very few studies were done in *Janthinobacterium sp.*

Secondary structural analysis

A secondary structure is formed from the interactions between near-by amino acids as the polypeptide begins to fold into its functional three-dimensional form. The secondary structure provides information about protein activity, relationships, and functions, this predicts the arrangement of catalytic residues in an active site, about a protein interacts with other proteins for structural or other regulatory purposes (Ma *et al*., 2018). Examining the conformational patterns of the secondary structure of the proteins of interest for VioA showed that all the *Janthinobacterium species* exhibited almost similar alpha helices whereas *Janthinobacterium sp B9_8* shows a different structural conformation having 15 alpha alpha helices and 14 beta sheets. *Janthinobacterium* and reference organism have almost similar secondary structure in VioB. VioB also showed similar structures. Structures of both the organisms revealed 16 alpha helices and 16 beta sheets. In VioC, the organisms were almost similar with 10 α-helices. In case of VioE, different structural architecture was identified. *Chromobacterium violaceum* showed 2 α-helices while the other *Janthinobacterium* species showed 2-4 α- helices at different regions.

Three-dimensional protein structure prediction

VioA protein structure prediction: *Chromobacterium violaceum* alone exhibited 99% similarity to the template. The *Janthinobacterium* species showed identity of 40-50% to the template 5ZBC with the resolution of 1.8 Å. The model was evaluated using Ramachandran plot and the predicted model was reasonably good (Figure: 4, AB). The results of VioB revealed poor identity of 19.85 % with Crystal structure of Ferritin like protein 3HL1 from *Caulobacter vibriodes.* Hence, the model is not reliable (Figure: 4, C). In VioC, *Janthinobacterium* species exhibited very poor identity of approximately around 30 % to 6GXS template indicating that the predicted model is of bad quality. In case of VioD, the reference organism showed 100% identity indicating the availability of the experimentally determined structure. *Janthinobacterium* species 3C4A showed 67% identity to the template and the Ramachandran plot revealed 93-95 % for the favourable region evaluating the predicted structure to be of a good quality. VioE *Janthinobacterium sp* 3BMZ showed identity of 52-60 % with template exhibiting resolution of 1.2 Å. The predicted structures of VioA, D and E are reasonably of good quality.

A recent study by Xu *et al*., 2019 confirmed that *Janthinobacterium B9_8* was different from the other species of *Janthinobacterium*. This study is in concordance with the earlier reports as revealed by this study, which also shows that *Janthinobacterium B9_8* is very similar to *Iodobacter fluviatilis*. Earlier study also reported that the efficiency of violacein from *Janthinobacterium B9_8* was 20 times higher than the other bacteria, exact reason for the increased efficiency could not be predicted. Sequence and structural analysis of the *vio* genes revealed that this species as a separate lineage from the other *Janthinobacterium* species.

Previous studies reported that the compound Violacein is highly efficient and has many useful properties. Pubchem database have shown various assays of Violacein among which antimalarial, antiparasitic activity against *Leishmania amazonesis* showed active results. Duran *et al*., 2007 suggested that Violacein showed activity against Polio after infection with HeLa cells. So, a docking study was performed to understand the activity against Polio virus capsid protein and the compound was also docked against Corona virus spike protein as an effort to predict the efficiency.

Toxicity testing

The violacein compound was analysed for its drug likeliness using SWISS ADME, Protox ADME, pkCSM. Drug likeness in SWISS ADME Lipinski rule showed 0 violation.Number of hydrogen bond in donor and acceptor according to SWISS ADME and pkCSM is 4, Octanole water partition coefficient is 1.37 LD50 value is 2800mg/kg with predicted toxicity class 5. pkCSM predicted the drug to be categorically hepatotoxic. As mentioned earlier, Duran *et al*., 2007 suggested that Violacein showed activity against Polio after infection with HeLa cells. With this results the compound was docked against Polio viral capsid and Corona virus spike proteins.

Docking analysis of violacein against Polio Virus

The polio virus structure retrieved from PDB: 6z6w (strain Saukett) stabilized virus which has a resolution of 3Å (Bahar *et al*., 2021). The active site determined using Scf bio server was visualized in PyMol and is shown in **Figure: 5A**. The output of Autodock Vina (Vina 2010) server for the Polio virus and Violacein compound showed 9 binding regions with best binding energy as -7.1 (kcal/mol). Visualization is essential to understanding structural biology (DeLano *etal*., 2004). It is predicted that the violacein compound bind to the active site of Polio virus and could be an efficient drug as (Figure: 5B and C).

Docking analysis of violacein against Corona Virus

The SARS-CoV-2 virus structure that was retrieved from PDB: 6moj with a resolution of 2.45Å (Lan *et al*., 2020). The active site determined using Scfbio server was visualized in PyMol and is shown in Figure: 5D. The output of Autodock Vina server (Vina. 2010*)* for the Corona virus and Violacein compound showed 9 binding regions with best binding energy of -8 (kcal/mol). The docked results revealed that the violacein compound bound to the protein (Figure: 5E). The hydrogen bond interaction is noted between the violacein compound as shown in Figure: 5F, but not in the active region. These results need to be further evaluated by wet lab-based methods.

CONCLUSION

Violacein a violet purple compound with various properties is a boon for its enormous significance in dyeing industry as an alternative to chemical dyes for violet colors in textile and food industry. It also has a major role in pharmaceutical industry for its wide range of antibacterial, antifungal, antiparasitic, antitumor and antiviral. The present sequence analysis revealed that the sequence variants did not affect the Violacein production in all organisms. This is possible because the conserved domain essential for structure-function relationship is observed in all the microbes irrespective of their lineage. *Janthinobacterium B9_8* Violacein gene and protein analysis is very similar to *Iodobacter fluviatilis* and different with other *Janthinobacterium species*. In addition, the violacein compound has successfully docked against Polio virus in its active site, but the compound showed interaction with the Corona virus. These results need to be confirmed with few more Corona viral proteins.

REFERENCES

1. Bahar, M. W., Porta, C., Fox, H., Macadam, A. J., Fry, E. E., & Stuart, D. I. (2021). Mammalian expression of virus-like particles as a proof of principle for next generation polio vaccines. *NPJ vaccines*, *6*(1), 1-11.
2. Malik, K., Tokkas, J., & Goyal, S. (2012). Microbial pigments: a review. *Int J Microbial Res Technol*, *1*(4), 361-365.

3. Bhoir, M., Ratnakaran, P., & Durve-Gupta, A. (2020). EXTRACTION OF ALGINATE BIOPOLYMER FROM ISOLATED AZOTOBACTER SPECIES AND ITS APPLICATIONS.
4. Balibar, C. J., & Walsh, C. T. (2006). In vitro biosynthesis of violacein from l-tryptophan by the enzymes VioA– E from Chromobacterium violaceum. *Biochemistry*, *45*(51), 15444-15457.
5. Batista, J. H., Leal, F. C., Fukuda, T. T., Alcoforado Diniz, J., Almeida, F., Pupo, M. T., & da Silva Neto, J. F. (2020). Interplay between two quorum sensing-regulated pathways, violacein biosynthesis and VacJ/Yrb, dictates outer membrane vesicle biogenesis in Chromobacterium violaceum. *Environmental microbiology*, *22*(6), 2432-2442.
6. Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, Schwede, T. (2014). SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic acids research*, *42*(W1), W252-W258.
7. Blum, Matthias, Hsin-Yu Chang, Sara Chuguransky, Tiago Grego, Swaathi Kandasaamy, Alex Mitchell, Gift Nuka et al. "The InterPro protein families and domains database: 20 years on." *Nucleic Acids Research* 49, no. D1 (2021): D344-D354.
8. Boggiano, B. G., Clarke, K., & TS, S. (1958). The chemistry of bacteria. Part VII. The structure of violacein. *Jour. Chem. Soc*, 755-760.
9. Choi, S. Y., Yoon, K. H., Lee, J. I., & Mitchell, R. J. (2015). Violacein: properties and production of a versatile bacterial pigment. *BioMed research international*, *2015*.
10. Canuto, J. A., Lima, D. B., de Menezes, R. R. P. P. B., Batista, A. H. M., Nogueira, P. C. D. N., Silveira, E. R. (2019). Antichagasic effect of violacein from Chromobacterium violaceum. *Journal of applied microbiology*, *127*(5), 1373-1380.
11. Combet, C., Blanchet, C., Geourjon, C., & Deleage, G. (2000). NPS@: network protein sequence analysis. *Trends in biochemical sciences*, *25*(3), 147-150.
12. de Carvalho, L. M., Fernandes, F. M., de Fátima Nunes, M., Brigola, J., Casbas, N., &Groves, C. (2013). History and cultivation of Parma Violets (Viola, Violaceae) in the United Kingdom and France in the nineteenth century. *Harvard Papers in Botany*, *18*(2), 137-145.
13. de Mejia, E. G., Zhang, Q., Penta, K., Eroglu, A., & Lila, M. A. (2020). The colors of health: Chemistry, bioactivity, and market demand for colorful foods and natural food sources of colorants. *Annual review of food science and technology*, *11*, 145-182.
14. DeLano, W. L., & Bromberg, S. (2004). PyMOL user's guide. *DeLano Scientific LLC*, *629*.
15. Di Muzio, E., Toti, D., & Polticelli, F. (2017). DockingApp: a user friendly interface for facilitated docking simulations with AutoDock Vina. *Journal of Computer-Aided Molecular Design*, *31*(2), 213-218.
16. Durán, M., Ponezi, A. N., Faljoni-Alario, A., Teixeira, M. F., Justo, G. Z., & Durán, N. (2012). Potential applications of violacein: a microbial pigment. *Medicinal Chemistry*
17. Federhen, S. (2012). The NCBI taxonomy database. *Nucleic acids research*, *40*(D1), D136-D143.
18. Ferreira, C. V., Bos, C. L., Versteeg, H. H., Justo, G. Z., Durán, N., & Peppelenbosch, M. P. (2004). Molecular mechanism of violacein-mediated human leukemia cell death. *Blood*, *104*(5), 1459-1464.
19. Finger, S., Godoy, F. A., Wittwer, G., Aranda, C. P., Calderón, R., & Miranda, C. D. (2019). Purification and characterization of indochrome type blue pigment produced by Pseudarthrobacter sp. 34LCH1 isolated from Atacama desert. *Journal of Industrial Microbiology and Biotechnology*, *46*(1), 101-111.
20. Füller, J. J., Röpke, R., Krausze, J., Rennhack, K. E., Daniel, N. P., Blankenfeldt, W,. & Moser, J. (2016). Biosynthesis of violacein, structure and function of l-Tryptophan oxidase VioA from Chromobacterium violaceum. *Journal of Biological Chemistry*, *291*(38), 20068-20084.
21. Geer, Lewis Y., Aron Marchler-Bauer, Renata C. Geer, Lianyi Han, Jane He, Siqian He, Chunlei Liu, Wenyao Shi, and Stephen H. Bryant. "The NCBI biosystems database." *Nucleic acids research* 38, no. suppl_1 (2010): D492-D496.
22. Gillis, M., & Logan, N. A. (2015). Janthinobacterium. *Bergey's Manual of Systematics of Archaea and Bacteria*, 1-12.
23. Giraldo-Calderón, G. I., Emrich, S. J., MacCallum, R. M., Maslen, G., Dialynas, E., Topalis, P., Lawson, D. (2015). VectorBase: an updated bioinformatics resource for invertebrate vectors and other organisms related with human diseases. *Nucleic acids research*, *43*(D1), D707-D713.

24. Hakvåg, S., Fjærvik, E., Klinkenberg, G., Borgos, S. E. F., Josefsen, K. D., Ellingsen, T. E., & Zotchev, S. B. (2009). Violacein-producing Collimonas sp. from the sea surface microlayer of costal waters in Trøndelag, Norway. *Marine drugs*, *7*(4), 576-588
25. Hirano, S., Asamizu, S., Onaka, H., Shiro, Y., & Nagano, S. (2008). Crystal structure of VioE, a key player in the construction of the molecular skeleton of violacein. *Journal of Biological Chemistry*, *283*(10), 6459-6466.
26. Hoshino, T. (2011). Violacein and related tryptophan metabolites produced by *Chromobacterium violaceum*: biosynthetic mechanism and pathway for construction of violacein core. *Applied microbiology and biotechnology*, *91*(6), 1463-1475.
27. Ibrahim, Y. M., Abouwarda, A. M., & Omar, F. A. (2020). Effect of kitasamycin and nitrofurantoin at subinhibitory concentrations on quorum sensing regulated traits of *Chromobacterium violaceum*. *Antonie van Leeuwenhoek*, *113*(11), 1601-1615.
28. Jiang, P. X., Wang, H. S., Zhang, C., Lou, K., & Xing, X. H. (2010). Reconstruction of the violacein biosynthetic pathway from Duganella sp. B2 in different heterologous hosts. *Applied microbiology and biotechnology*, *86*(4), 1077-1088.
29. Karsch-Mizrachi, I., Takagi, T., Cochrane, G., & International Nucleotide Sequence Database Collaboration. (2018). The international nucleotide sequence database collaboration. *Nucleic Acids Research*, *46*(D1), D48-D51.
30. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, Bolton, E. E. (2019). PubChem 2019 update: improved access to chemical data. *Nucleic acids research*, *47*(D1), D1102-D1109.
31. Kleinjung, J., Douglas, N., & Heringa, J. (2002). Parallelized multiple alignment. *Bioinformatics*, *18*(9), 1270-1271.
32. Konzen, M., De Marco, D., Cordova, C. A., Vieira, T. O., Antonio, R. V., & Creczynski-Pasa, T. B. (2006). Antioxidant properties of violacein: possible relation on its biological function. *Bioorganic & medicinal chemistry*, *14*(24), 8307-8313.
33. Koonin, E., & Galperin, M. Y. (2002). Sequence—evolution—function: computational approaches in comparative genomics.
34. Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., ... & Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, *581*(7807), 215-220.
35. Lellis, B., Fávaro-Polonio, C. Z., Pamphile, J. A., & Polonio, J. C. (2019). Effects of textile dyes on health and the environment and bioremediation potential of living organisms. *Biotechnology Research and Innovation*, *3*(2), 275-290.
36. Lopes, S. C., Blanco, Y. C., Justo, G. Z., Nogueira, P. A., Rodrigues, F. L., Goelnitz, Costa, F. T. (2009). Violacein extracted from Chromobacterium violaceum inhibits Plasmodium growth in vitro and in vivo. *Antimicrobial agents and chemotherapy*, *53*(5), 2149-2152.
37. Lyakhovchenko, N. S., Abashina, T. N., Polivtseva, V. N., Senchenkov, V. Y., Pribylov, D. A., Chepurina, A. A., ... & Solyanikova, I. P. (2021). A Blue-Purple Pigment-Producing Bacterium Isolated from the Vezelka River in the City of Belgorod. *Microorganisms*, *9*(1), 102.

Table: 1 Organisms employed in this study and corresponding Genbank Id

SI.NO	Organism	Genbank Id
1	*Pseudoalteromonas luteoviolacea*	GCA_001750165.1
2	*Pseudoalteromonas tunicata*	GCA_002310815.1
3	*Chromobacterium violaceum*	GCA_000007705.1
4	*Chromobacterium vaccinii*	GCA_001855275.1
5	*Chromobacterium phragmitis IIBBL 274-1*	GCA_003325475.1
6	*Chromobacterium paludis*	GCA_008275125.1
7	*Iodobacter fluviatilis*	GCA_004194535.1
8	*Janthinobacterium agaricidamnosum*	GCA_000723165.1
9	*Janthinobacterium sp B9_8*	GCA_000969645.2

10	*Janthinobacterium sp. 1_2014MBL_MicDiv*	GCA_001865675.1
11	*Janthinobacterium sp. LM_6*	GCA_002002885.1
12	*Janthinobacterium lividium*	GCA_013372045.1
13	*Massilia sp. NR 4_1*	GCA_00119105.1
14	*Massilia violaceinigra*	GCA_002752675.1
15	*Myxococcus stipitatu*	GCA_000331735.1
16	*Rivularia sp. PCC 7116*	GCA_000316665.1

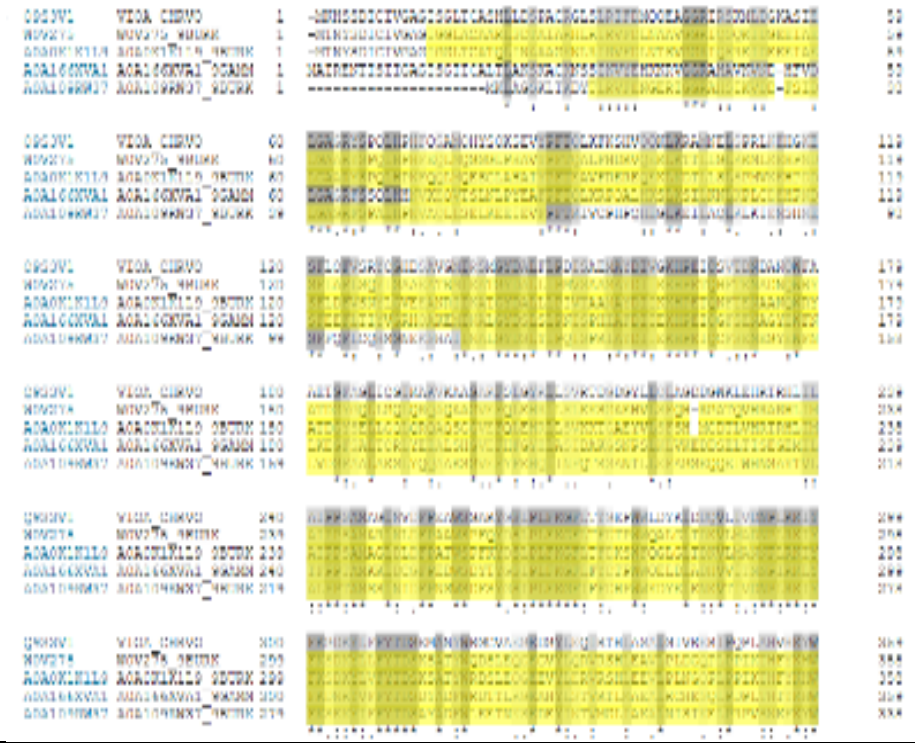
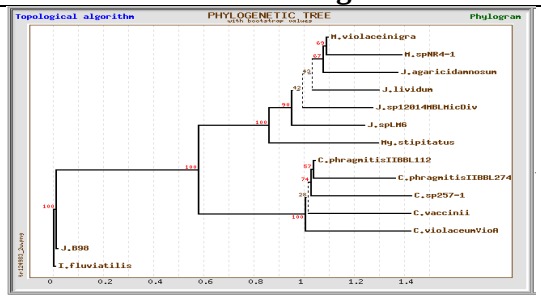
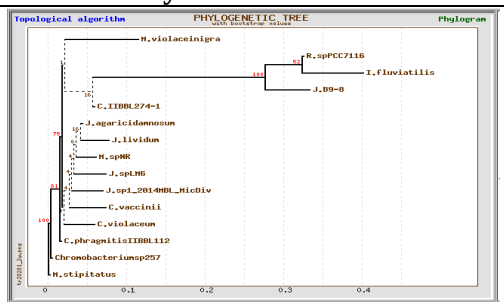


Figure : 1 Conserved Domain analysis



A



B

Figure: 2 Phylogenetic analysis

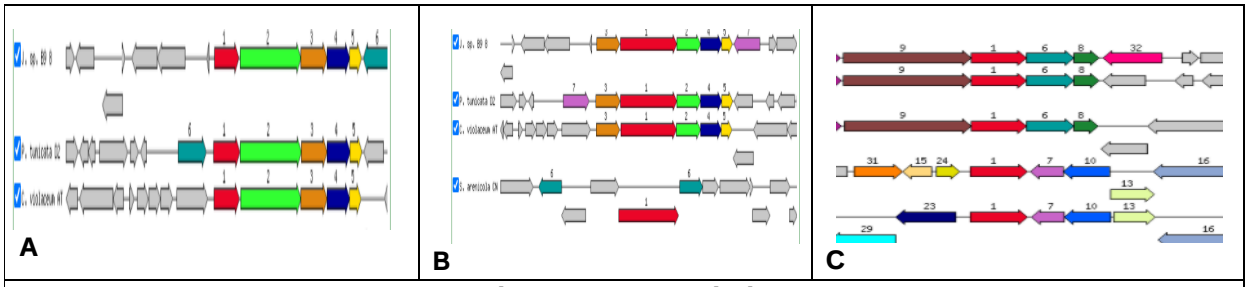


Figure: 3 RAST analysis

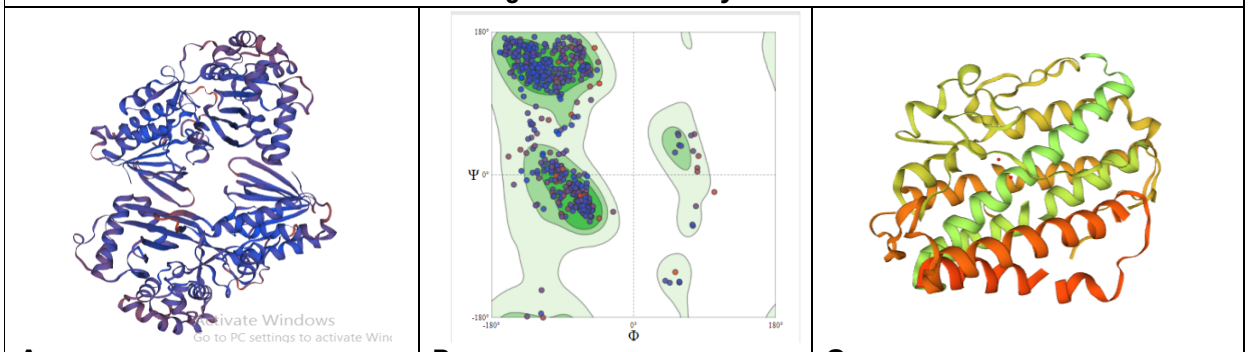


Figure: 4 Tertiary structure analysis

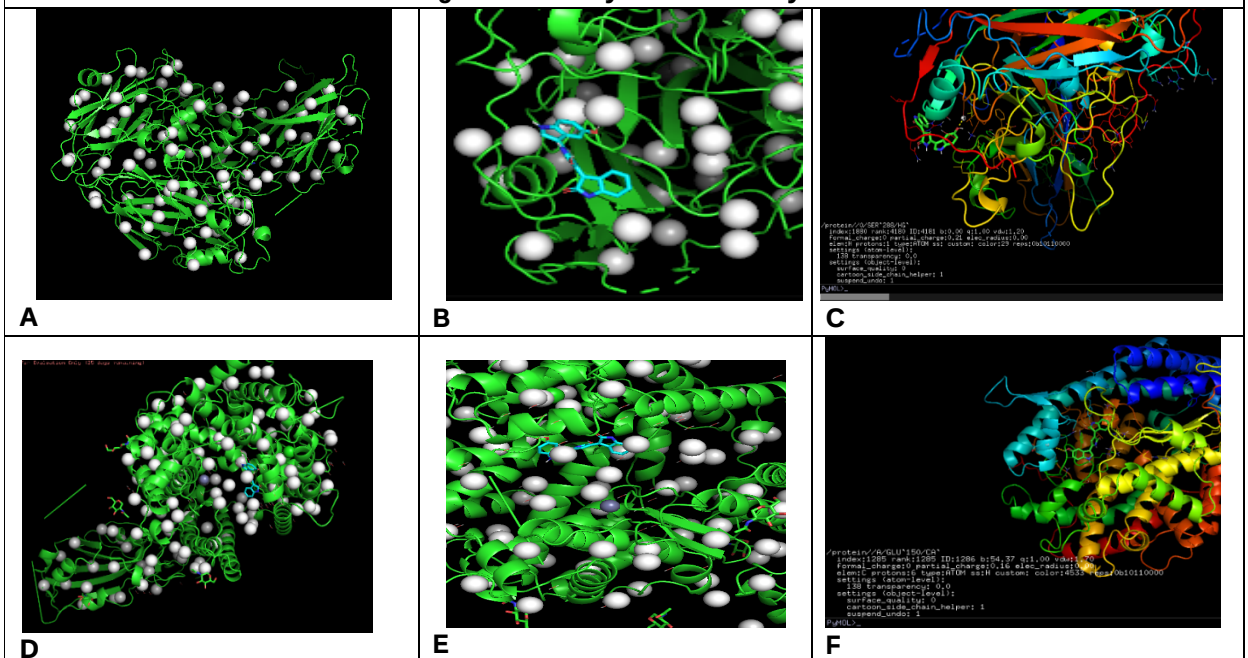


Figure: 5 Docking studies

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REVIEW ARTICLE

Review on Physico-Chemical Characterisation of Coffee Cherry Pulping Waste Water

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ABSTRACT

Coffee effluent is a wastewater from coffee industry after the coffee cherry pulping process by wet pulping method. *Coffea arabica L.* is predominantly cultivated coffee bean in Western Ghats area in India. The physico chemical characteristic of coffee effluent is reviewed from previous studies. The effluent is brown in color due to the presence of tannins and flavonoids. The total solid content of the wastewater ranges between 6400-6800mg/L, The total dissolved solids ranges between 4000-5000 mg/L. Generally TDS reflects in the color, taste and odor of the effluent. The Total suspended solids fluctuated from 1400 mg/L to 2000 mg/L. The effluent had acidic fruity odor which is objectionable and the pH is 3.69. Dissolved oxygen level was reported as zero in the effluent. The chemical parameters like chloride, fluoride, phosphate, sulphate and nitrate in most of the samples were 150 mg/L, 6 mg/L4 mg/L, 409 mg/L and 38 mg/L respectively. The BOD was between 14000-17000mg/L and the COD ranges between 27000-30000 mg/L. The amount of BOD and COD is higher in coffee effluent due to the decomposition of organic matters in the wastewater. The higher amount of biological and chemical oxygen demand will affect the aquatic life. These reported parameters have significant reasons for its existence in the effluent and insists to be removed. The physico-chemical characterization is a powerful tool to understand the nature and behavior of pollutants in the effluents and the need of its treatment before discharging it in to the surrounding environment.

Keywords: physico-chemical, coffee effluent, wastewater processing, CCPWW, characterization

INTRODUCTION

The word coffee came from an Arabic word *Qahweh* refers to a type of wine later became *Kahveh* in Turkish and Koffie in Dutch. Coffee is widely consumed, sweet smelling and energizing refreshment. Coffee is the world's most valuable traded commodity next to petroleum (Murthy and Naidu., 2012). Multiple countries tried to ban coffee but

all gone in vain. Research says that the people who drinks four cups of coffee in a day tends to live longer with reduced risk of CVD(Cardio Vascular Disease), DM II (Diabetes Mellitus) and Parkinson's. Studies have also shown that drinking coffee may lower the risk of depression. It is rainfed crop and grows in ecologically vulnerable eco system. Among 60 species in coffea genus, *Coffee arabica L.* and *Coffea robusta* alone dominates international trade. The coffee fruit phenotypically resembles a cherry and hence named "coffee cherry" (Wilbaux., 1956).India ranks sixth in global coffee cultivation. The topmost region of coffee cultivation in India has been identified by the coffee board of India depending on its taste and climate. In Tamilnadu, Nilgiris, Yercaud and Kodaikanal are the coffee cultivating regions.

Characteristics of Coffee

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Rubiales
Family	:	Rubiaceae
Genus	:	Coffea L.
Species	:	*Coffea arabica L.*

Robusta coffee has more acidic flavor and higher level of caffeine. Arabica is known for its delicate flavor and low acidity. Caffeine contains xanthine compounds which results in its bitter flavor (Bolton and Null., 1981). Coffee accelerates metabolism, and it takes a day to fully eliminate caffeine from your system. Despite the fact that coffee is a mild diuretic, hence it keeps people hydrated. The aqueous solubility of caffeine is metabolized by liver and 0.5-10 % is excreted through urine and feces. (Knee *et al*., 2010)

Processing of coffee

The way of processing determines the quality of end product (Alfaro and Rodriguez., 1994).The ripe coffee cherry process several steps for the exfoliation of the outer and internal husk, mucilage, silver skin and parchment to obtain clean, healthy, and quality coffee beans. Unripe coffee cherries are very tough to process and produce a low-quality coffee bean. Coffee cherry processing includes wet and dry method (FDRE., 2006). Wet processing provides high-quality coffee beans compared with dry processing but costlier when compared with dry processing (Pandey *et al*., 2000). Enormous amount of water is utilized in the processing of coffee cherry. About 40-45 L of wastewater is produced when pulped 1 kg of coffee cherry. When calculated annually the waste generation is of million tons in both wet and dry processing methods (Fan *et al*., 2003). Wet method is widely used for *Coffea arabica L* cherries. 80,000L of water is used to clean 1 ton of *Coffea arabica* cherries and 93,000 L for *Coffea robusta* cherries by using conventional cherry pulper and washer (Shanmukhappa *et al*., 1998)

The Discharge standards for coffee processing waste by the CPCB as follows

In studies the pH and COD values were compared against the standards of Indian CPCB for coffee effluent discharge norms and other parameters are compared with common effluent discharge limits.

COLOR

The effluent is brown in color due to presence of tannin and caffeine. The intensity of color varies with different pH. Effluent with pH 7 showed dark green or black color due to the presence of flavonoid which prevents the penetration of sunlight into water (Eden., 2002). The coffee cherry pulping wastewater was rich in tannin, protein, alkaloids, cellulose and lignin (Deepa *et al*., 2002), which results in composition and the color of the effluent (Murthy and Naidu., 2012). The color of the effluent indicates it as a visible pollutant which is unfit for utilization (Fazli *et al*., 2010). The brown shade of the effluent is because of tannin, flavonoids and phenolic compounds (Fia *et al*., 2008). Many studies reported brown color effluent in common.

ODOUR

The effluent had acidic fruity odor which is objectionable. The strength of liberated gas results in the intensity of odor. It may be due to degeneration of flavonoids and pectin substances on fermentation the effluent gives acidic fruity smell (Shivap *et al*., 2008). Hariprasad (2013) has reported offensive odor in CCPWW.

pH

The pH is generally acidic and ranges from 3.5 to 4. Roger (1994) has clearly explained that any fluids overloaded with hydrogen ions turns the solution acidic. When the fermentation of organic matter occurs simultaneously pH of effluent also increases and turns the effluent more acidic (Mburu *et al*., 1994). Studies of Ancy(2020), Mburu(1994), Hue(2006) was compared. Irrespective of difference in time frame, place and sampling site, the effluent showed pH range with 3.5-4.5. Added this acidic range is because of organic contribution such as Chlorogenic acid and Quinic acid from coffee cherries. (Hue *et al*., 2006)

TEMPERATURE

Researchers stated that the temperature of effluent has no way affected the environment. The temperature analyzed by Ancy (2020) was 22°C at 1500m above mean sea level. Devi *et al* (2008) has reported 25°C in zimma zone Ethiopia. Emile *et al* (2020) has reported 23°C in coffee growing ecological zones in Burnundi.

TOTAL SOLIDS

The dissolved and suspended particles together cause water pollution is termed as solids. The total solid content of the wastewater was 6400-6800 mg/L, The total dissolved solids range was 4000 - 5000 mg/L. Generally TDS reflects in the color, taste and odor of the effluent. The Total suspended solids fluctuated from 1400 mg/L to 2000 mg/L. Higher TDS causes osmotic stress that disturbs aquatic freshwater living organisms and reduce the potability of water (Teckle *et al*., 2013) TDS comprises both organic and inorganic substance. Ancy *et al*., 2020 stated the higher TDS is because of dissolved mucilage which forms crust on the effluent.

DISSOLVED OXYGEN

The acidic nature of coffee cherry pulping wastewater reduces the oxygen level in effluent (Freshner and Schitzer., 1996). The higher concentration of solids in CCPWW is biodegradable, Hence the dissolved oxygen is decreased (Shanmukhappa *et al*., 1998). Many researchers have reported the Dissolved oxygen level was zero in many coffee effluent samples.

BIOLOGICAL AND CHEMICAL OXYGEN DEMAND

The BOD is used to oxidize the natural organic matter and organic waste in the water. COD is used to measure the amount of oxygen required to oxidize the organic matter in wastewater, which produces carbon di oxide and water. The BOD was 14000-17000 mg/L and the COD was 27000-30000mg/L. The amount of BOD and COD are higher in coffee effluent due to the decomposition of organic matters in the wastewater and presence of high solid content (Nelson *et al*., 2009). The increased biological and chemical oxygen demand is not suitable for aquatic life and on exposure of air the color changes to black that denotes the effluent cannot be bio degraded. BOD and COD are found to be higher than the CPCB standards. Gray(2004) has reported BOD and COD together urge to oxygen demand for pollution degradation. The blending of BOD and COD tests, favor the indication of toxic conditions and biological resistance in wastewater (Devi *et al*., 2002).

CHEMICAL PARAMETERS

The chemical parameters like chloride, fluoride, phosphate, sulphate and nitrate in the effluent reported in many papers were 150 mg/L, 6 mg/L, 4 mg/L, 409 mg/L, and 38 mg/L respectively. The concentration of chloride, fluoride, phosphate, sulphate and nitrate were also observed to be low. Fluoride is essential for human tooth formation and has remineralizing power of tooth enamel which prevents from dental plague (Trivedy and Goel., 1986). Chloride presence is due to dissolution of calcium and potassium.

Phosphates and Nitrates are essential for aquatic plants and taken by them, they together reported as plant nutrients. If the concentration of phosphates and nitrates are high it disturbs the aquatic ecosystem and cause eutrophication, be a threat for organisms in it. The level of phosphate and nitrate reflects in the BOD of effluent indirectly (Trivedy and Goel., 1986)

Effects of coffee effluent

Coffee processing industries produces very high pollution load wastewater, they produces large amount of wastewater that contains higher concentration of organic matter. Discharge of effluent into environment reduces the availability of potable water. The untreated effluent is directly discharged into the nearby water bodies, streams and open land causing severe illness such as skin irritation, nausea, giddiness and breathing problems. The effluent precipitates on water bodies when exposed to atmospheric air thus threatens the aquatic systems. Effluent on direct discharge exhibits the nature of acidic fruity odor because of the presence of organic solids. The components of effluent has lethal nature and leads to ecological contamination (Pandey *et al*., 2001).

CONCLUSION

The Physico chemical properties of coffee effluent emphasize the necessity of treatment in order to prevent the environmental pollution. According to many researchers, higher TDS, BOD, COD and acidic pH showed the contamination of ground water near coffee hub because of the open discharge of coffee cherry pulping wastewater. The higher TDS and COD prove the toxicity of effluent hence it shouldn't be discharged directly into the environment. These parameters has significant reasons for its existence in the effluent and insists to be removed. The physico chemical characterization is a powerful tool to understand the nature and behavior of pollutants in the effluents and the need of its treatment before discharging it in to the surrounding environment. Removal of pollutants from the effluent by an eco-friendly technique is need of hour. It will be the best and effective methods as it has no side effects and will not produce any secondary pollutants.

ACKNOWLEDGEMENT

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REFERENCES

1. Ancy Jenifer A, M. Vasanthy, C. Thamarai selvi, B. Ravindran, Woo Jin Chung, Soon Woong Chang(2020). Treatment of coffee cherry pulping wastewater by using lectin protein isolated from *Ricinus communis* L. seed. Journal of Water Process Engineering
2. Bolton S, Null G (1981) Caffeine, psychological effects, use and abuse. Orthomolecular psychiatry 10(3), 202 –211.
3. Deepa GB, Chanakya HN, Dealwis AAP, Manjunath GR, Devi V (2002) Overcoming pollution of lakes and water bodies due to coffee pulping activities with appropriate technology solutions. In: Proceedings Symposium on Conservation, Restoration and Management of Aquatic Ecosystems, paper 4.
4. Devi R, Dahiya RP, Gadgil K (2002) Investigation of coconut coir carbon and sawdust based adsorbents for combined removal of COD and BOD from domestic wastewater. Water Environmental Managment Service. International Water Association, 1209–1218.
5. Fazli M, Mesdaghinia AR, Naddafi K, Nasseri S, Yunesian M, Mazaheri Assadi M, Rezaie S, Hamzehei H, (2010). Optimization of reactive blue19 decolorization by *Ganoderma sp*. using response surface methodology. Iranian Journal of Environmental Health Science Engineering, 7(1), 35- 42.

6. Federal Democratic Republic of Ethiopia (FDRE) (2006) Unpublished Statistics. Addis Ababa: Federal Democratic Republic of Ethiopia, Ministry of Agriculture and Rural Development.
7. Fia FRL, Matos AT, Borges AC, Fia R, Cecon PR (2012) Treatment of wastewater from coffee bean processing in anaerobic fixed bed reactor with different support materials: performance and kinetic modeling. Journal of Environmental Management 108, 14-21.
8. Fresner J, Schnitzer H (1996) How coffee-making can help one understand cleaner production. Journal of Cleaner Production, 4 (3-4), 213–217.
9. Haddis A. and Devi R. (2008). Effect of effluent generated from coffee processing plant on the water bodies and humanhealth in its vicinity. Journal of Hazardous Materials. 152:259-262.
10. Hariprasad NV, Samanvitha N, Ayisha Tasneem, Priyanka M, Rakshitha A, Dayananda HS (2013) Eco-engineered treatment of coffee processing wastewater. International Journal of Engineering Research and Science Technology, 2(3), 97-101.
11. http://cpcb.nic.in/uploads/Industry-Specific-Standards/Effluent/35-coffee.pdf
12. Hue NV, Bittenbender HC, Ortiz-Escober ME (2006) Managing coffee processing water in Hawaii. Journal of Hawaiian Pacific Agricriculture, 13, 15-21.
13. KL, Gossett R, Boehm AB, Paytan A (2010) Caffeine and agriculture pesticides concentrations in surface water and groundwater on the North Shore of Kauai (Hawaii, USA). Marine Pollution Bulletin 60(8), 1376–1382.
14. L, Soccol AT, Pandey A, Soccol CR (2003) Cultivation of *Pleurototus* mushroom on Brazilian coffee husk and effects of caffeine and tanninc acid. Micologia Aplicada International, 15, 15-21.
15. Mburu, J.K., Thuo, J.T., Marder, R.C., (1994). Thecharacterization of coffee wastewater from coffee processing factories in Kenya. Kenya Coffee 59, 1756- 1763.
16. MR, Rodríguez JJ (1994) Impacto ambiental del procesamiento del café en Costa Rica. Agronomía Costarricense, 18(2), 217-225.
17. Murthy PS, Naidu M (2012) Sustainable management of coffee industry by-products and value addition-A review. Resources, Conservation and Recycling, 66, 45–58.
18. Nelson N, Franklin A, Patrick S, Joseph S (2009) Environmental engineering, water, wastewater, soil and Groundwater treatment and remediation. 6th edition, John Wiley & Sons, Inc.
19. Novita E (2016) Biodegradability simulation of coffee wastewater using instant coffee. Agriculture and Agricultural Science Procedia 9, 217 – 229.
20. Padmapriya R, Tharian JA, Thirunalasundari (2015) Treatment of coffee effluent by- *Moringa oleifera* seed. International Journal of Current Microbiology and Applied Sciences, 4(1), 288-295.
21. Pandey A, Soccol C, Nigam P, Brand D, Mohan R, Roussos S (2000) Biotechnological potential of coffee pulp and coffee husk for bioprocesses. Biochemical Engineering Journal, 6, 153-162. .
22. Roger N (1994). Environmental Analysis. University of Greenwich, John Wiley and Sons.
23. SA, Reinhardt HW (2010) Fibres of coffee husk and hulls for the production of particle board. Materials and Structures, 43, 1049–1060
24. Selvamurugan, M., P. Doraisamy, M. Maheswari and N.B. Nandakumar (2010) High rate anaerobic treatment of coffee processing wastewater using up flow anaerobic hybrid reactor. Iran Journal of Environmental Health Science and Engineering, 7(2), 129-136.
25. Shanmukhappa DR, Ananda Alwar RP and Srinivasan CS (1998), Water pollution by coffee processing units and its abatement. Indian Coffee, 10, 3-9.
26. Shivap PP, Jay A, Mahadevan KM (2008) Conjunctive use of coffee effluent and fresh water on performance of young *Arabica coffee*. Journal of Plant crop 36, 30-37.
27. ThamaraiSelvi C, Rajalakshmi SB, Jenifer AA, Ahila KG, Nithya A (2014) Effective Removal Of Nitrate From Potable Water Of Kodaikanal Hills Using Natural Coagulant. International Journal of Informative & Futuristic Research, 2(1), 92-104.
28. Trivedi RK, Khatavkar SB, Goel PK (1986) Characterisation, treatment and disposal of wastewater in a textile industry. Industrial Pollution Control, 2 (1), 1 – 12.
29. WHO (1995), Guideline for Discharge of Industrial Effluent Characteristics, vol.3, pp. 231–236.

30. Wilbaux R (1956) Les caféiers au Congo Belge. Technologie du café Arabica et Robusta, Publication de la direction de l'Agriculture, des Forets et de l'élevage. Bruxelles, 213.

Table 1. Physico chemical characterization

S.No	PARAMETERS	OBSERVATION	CPCB STANDARD
1	Color	Brown	Colorless
2	Odor	Objectionable, Fruity	Odorless
3	pH	3.5-4	6.5-8.5
4	Temperature	22°C	Shall not exceed above 5°C above the receiving water temperature
5	Total Solids	6400-6800 mg/L	-
6	Total dissolved Solids	4000-5000 mg/L	2100 mg/L
7	Total suspended solids	1400-2000 mg/L	100-600 mg/L
8	Dissolved Oxygen	0	-
9	Biological Oxygen Demand	14000-17000 mg/L	1000 mg/L
10	Chemical Oxygen Demand	27000-30000 mg/L	250 mg/L
11	Chloride	120-150 mg/L	600-1000 mg/L
12	Fluoride	4-8 mg/L	2-15 mg/L
13	Phosphate	3-4mg/L	5 mg/L
14	Sulphate	400-500 mg/L	1000 mg/L
15	Nitrate	30-38 mg/L	10-20 mg/L

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RESEARCH ARTICLE

Fourier Transform Infrared Spectral Analysis of *Furcraea foetida* Root Extracts

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ABSTRACT

Furcraea foetida (L.) Haw. also known as giant cabuaya or green-aloe is a monocot plant of Asparagaceae family. The root of the plant was extracted using various solvents of varying polarity and the extracts were subjected to Fourier transform infrared (FTIR) spectroscopy analysis to characterize the bioactive compounds. This spectroscopy technique helps with the preliminary identification of the chemical constituents and structures of the compounds present in plant extracts. The FTIR spectrums of the hexane and chloroform extracts showed 6 peaks each whereas only 5 and 4 peaks were found in the acetone and methanol extracts. The FTIR analysis confirmed the presence of alcohols, phenols and alkenes in most extracts. The results of this analysis confirms the presence of bioactive phytocompounds.

Keywords: *Furcraea foetida*, FTIR, spectroscopy, phytocompounds

INTRODUCTION

Furcraea foetida (L.) Haw. is a member of the sub-family Agavoideae and family Asparagaceae according to the Angiosperm Phylogeny Group III system, 2009 [1]. It is commonly known as Mauritius hemp. This plant is of economic value due to the presence of rough fibres. This plant is native to the Caribbean and Northern South America and has been naturalized in many countries including India. A few cultivars including variegated varieties are grown as ornamental plants in various parts of the world. *F. foetida* is used for its anti-inflammatory and anti-tumour properties [2]. Dispersive spectrometer measures intensity over a narrow range of wavelengths at a time whereas a FTIR spectrometer collects high spectral resolution data over a wide spectral range simultaneously. Fourier Transform Infrared Spectrophotometer (FTIR) is an efficient tool used for identifying the types of chemical bonds or functional groups of compounds present in plant extracts. The main objective of the present study is to identify the phytoconstituents of various extracts of *F. foetida* root by FTIR analysis.

MATERIALS AND METHODS

Plant Collection

Furcraea foetida plants were collected from Perumalmalai, Kodaikanal, Tamil Nadu. The roots of the plants was separated and cut into small pieces. They were washed thoroughly in tap water thrice to remove dirt and then were rinsed with distilled water. The roots were pat dry and dried in a hot air oven at 45ºC for 2 days. The dried roots were powdered using mechanical grinder. The powder was then sieved for removal of fibers and stored in air tight containers at room temperature for further use.

Extract preparation

Extracts of the roots of *F. foetida* were prepared by mixing 1g of the powdered plant sample with 50 ml of organic solvents such as hexane, acetone, chloroform and methanol in separate conical flasks at room temperature for 2 days. Later for 45 minutes the mixture was heated at 40°C in a water bath. The solutions were filtered through Whatman No. 1 filter to remove residues.

FTIR analysis

FTIR analysis of leaf and root powders were carried out using Shimadzu FTIR spectroscope (Shimadzu, IR Affinity 1, Japan). A small quantity of extract was mixed with KBr in order to prepare translucent sample discs. The disc was loaded in FTIR spectroscope, with a scan range from 400 to 4000 cm^{-1} with a resolution of cm^{-1}.

RESULTS AND DISCUSSION

The FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition [3]. Based on the peak value in the region of infrared radiation, the FTIR spectrum was used for the identification of the functional groups of the bio-active compounds present in the samples. The hexane extract of root gave the following characteristic absorption peaks as represented in the Figure 1. A total of 6 peaks of frequencies 3444.26, 2923.30, 1638.42, 1456.37, 1176.50 and 720.97 were found in the spectrum. The respective functional groups and bonds for the respective frequencies are listed in Table 7. Figure 2 shows the 5 characteristic absorption peaks of the acetone of root. Alcohols, phenols, alkanes, aromatics, aromatics, aliphatic amines and alkynes compounds were found to be present in the extract. The respective functional bonds for the respective frequencies are listed in Table 2.

Figure 3 represents the 6 characteristic absorptions shown by the root chloroform extract. In Table 3 the respective functional groups and bonds for the 3445.48, 2925.56, 1640.22, 1481.16, 1104.49 and 635.17 frequencies are listed. The methanolic extract gave the following characteristic absorption peaks as represented in the Figure 4. A total of 4 peaks of frequencies 3425.86, 1655.04, 1066.35 and 1006.28 were found in the spectrum. The respective functional groups and bonds for the respective frequencies are listed in Table 4. Spectroscopic method like FTIR can be used separate in this sense as well as conventional methods [4]. An infra-red spectrum acts similar to a fingerprint of a sample as the absorption peaks directly corresponding to the frequencies of vibrations between the bonds of the atoms which makes up the plant material [5][6][7][8][9]. The size of the peaks in a spectrum is directly correlative to the quantity of the compound [10]. Infrared spectroscopy can be used for positive identification of compounds. This is due to the fact that no two compounds produce the exact same infrared spectrum thus making them unique.

CONCLUSION

The results help in analysing the phytochemistry of the root of the plant *F. foetida* and confirm the fact that it contains important bioactive phytocompounds. FTIR analysis showed the presence of alcohols, phenols, alkanes, aldehydes,

alkyl halides, primary amines and aromatic compounds which could contribute to the various medicinal properties of this plant.

REFERENCES

1. P.F. Stevens. 2001onwards. Angiosperm PhylogenyWebsite. http://www.mobot.org/MOBOT/research/APweb/. Version 14, July 2017.
2. V. Nandagopalan , A. Doss, S.P. Anand. "An ethnobotanical study in the Pudukkottai district, South India". *The International Journal of Science and Technoledge*. 2014 Aug 1;2(8):1.
3. Y.C Du. Study of vermifuges. I. "Isolation of embelin from the fruit of Mugua-wha (*Embelia oblongifolia* Hemsl.)". *Yao Xue Xue Bao*. 1963;10:578-80.
4. M. Ibrahim , A.J. Hameed , A. Jalbout. "Molecular spectroscopic study of River Nile sediment in the greater Cairo region". *Applied spectroscopy*. 2008 Mar;62(3):306-11.
5. O.P. Sati, G. Pant. "New furostanol glycosides from *Asparagus plumosus* Leaves". *Journal of natural products*. 1985 May;48(3):390-4.
6. M.M. Rawat, D.S. Negi, M.S. Panwar, G. Pant , S. Shibata. "Anthraquinone glycosides from *Rheum moorcroftianum* Royle". *Pharmazie*. 1989;44(7):509-10.
7. M.C. Purohit, G. Pant, M.S. Rawat. "A betulinic acid glycoside from *Schefflera venulosa*". *Phytochemistry*. 1991 Jan 1;30(7):2419.
8. R.K Joshi, D. Prasad, M.S. Rawat, G. Pant. "Allelopathic effect of aqueous extracts of leaves of *Fraxinus micrantha* L. on crops". *Allelopathy Journal*. 1996;3(2):255-60.
9. D. Prasad, R.K. Joshi, G. Pant, M.M. Rawat. "Ephedrannin A from *Prunus armeniaca* root". *Fitoterapia* (Milano). 1997;68(3):275-6.
10. S. Pramod, H.C. Andola, M.S. Rawat, G.J. Pant, V.K. Purohit. "Fourier transform infrared (FT-IR) spectroscopy in an-overview". *Research Journal of Medicinal Plant*. 2011;5(2):127-35.

Table 1. Functional groups of biomolecules found in hexane extract of *Furcraea foetida* root

S.No	Frequency	Bond	Functional group
1.	3444.26	O–H stretch, H–bonded	Alcohols, phenols
2.	2923.30	C–H stretch	Alkanes
3.	1638.42	N–H bend	1° amines
4.	1456.37	N–O asymmetric stretch	Nitro compounds
5.	1176.50	–CH 2 X	Alkyl halides
6.	720.97	C–H rock	Alkanes

Table 2. Functional groups of biomolecules found in acetone extract of *F. foetida* root

S.No	Frequency	Bond	Functional group
1.	3442.38	O–H stretch, H–bonded	Alcohols, phenols
2.	1631.78	N–H bend	1° amines
3.	1253.37	C–O stretch	Alcohols, carboxylic acids, esters, ethers
4.	1137.63	C–N stretch	Aliphatic amines
5.	633.27	C–Br stretch	Alkyl halides

Table 3. Functional groups of biomolecules found in of chloroform extract of *F. foetida* root

S.No	Frequency	Bond	Functional group
1.	3445.48	O–H stretch, H–bonded	Alcohols, phenols
2.	2925.56	C–H stretch	Alkanes
3.	1640.22	–C=C– stretch	Alkenes
4.	1481.16	C–C stretch (in–ring)	Aromatics
5.	1104.49	C–N stretch	Aliphatic amines
6.	635.17	C–Br stretch	Alkyl halides

Table 4. Functional groups of biomolecules found in methanol extract of *F. foetida* root

S.No	Frequency	Bond	Functional group
1.	3425.86	O–H stretch, H–bonded	Alcohols, phenols
2.	1655.04	–C=C– stretch	Alkenes
3.	1066.35	C–O stretch	Alcohols, carboxylic acids, esters, ethers
4.	1006.28	C–O stretch	Alcohols, carboxylic acids, esters, ethers

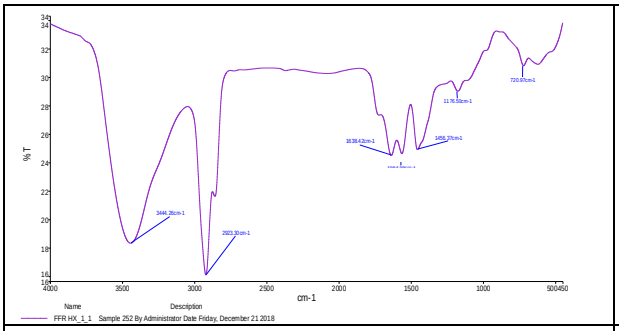

Fig 1. FTIR spectrum of hexane extract of *Furcraea foetida* root

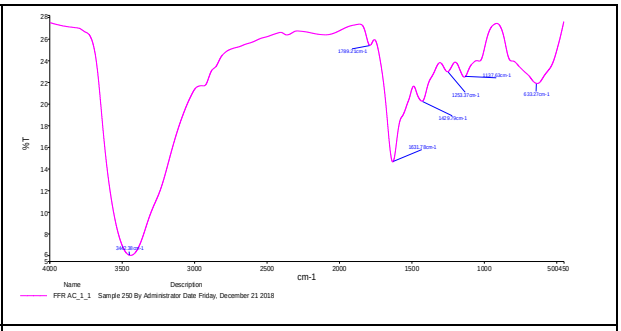

Fig 2. FTIR spectrum of acetone extract of *F. foetida* root

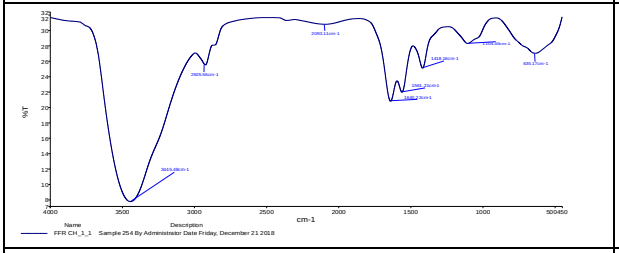

Fig 3. FTIR spectrum of chloroform extract of *F. foetida* root

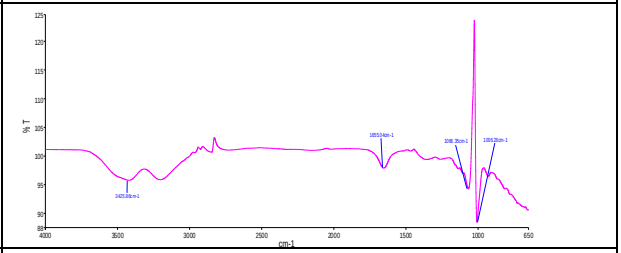

Fig 4. FTIR spectrum of methanol extract of *F. foetida* root

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RESEARCH ARTICLE

Molecular Docking Analysis of *Ficus carica* Linn. against ACE2 Receptors - PDB – 2AJF of Covid 19

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ABSTRACT

Corona virus disease 2019 (COVID-19) pandemic has created a sudden significant increase in hospitalization. Ever since the outbreak of COVID-19, there has been a barrage of health researches to curtail its spread and its harmful effects. SIDDHA system of medication, is one of the complimentary system of drugs that's practiced in South India since centuries. The SIDDHA system is deferentially considered as KARPAGA VRIKSHA, a desired tree as at holds in its majestic vastness - all things coherent & empirical by man. However, the medical dimension is taken into account as its first vital door to the current mystic tradition and shines as its hallmark. In SIDDHA literatures, *Ficus carica* (Aththi) is understood for its medicinal values and it's utilized in many siddha herbal formulations. Recent research states that, the Corona virus uses special surface glycol protein called spike to attach to ACE2 and intrude the hosting cells (LETKO et.al 2020). The density of ACE2 in each tissue, correlates with the severity of the disease therein tissue. And it has been suggested that decreasing ACE2 activity could be protective (Zhang et.al 2020, Zheng et.al 2020). So during this study, *Ficus carica* bark is subjected to molecular docking analysis to research its ACE2 receptor inhibiting property.

Key words: SIDDHA, *Ficus carica*, Molecular Docking, ACE2 Receptor, PDB.

INTRODUCTION

Molecular docking is a virtual screening method that predicts how drug candidates bind to a receptor of known 3D structure generating a score (binding energy value) that defines binding affinities between ligand and target protein. Molecular docking is becoming a robust tool within the discovery of drug candidates. RNA dependent RNA polymerase, main protease and angiotensin converting enzyme 2 receptors are a number of the targets available for testing the efficacy of the disease. Knowledge of microbes and therefore the disease spread is clearly mentioned in SIDDHA, which is evinced by "kirumiyal vantha thodam peruga undu" (lines mentioned in guru naadi)Various parts of thefig plant like bark, leaves, tender shoots, fruits, seeds and latex are medicinally important. In this study Ficus carica is subjected to docking analysis

OBJECTIVE

Binding of phytocomponents with the core amino acids (31 LYS and 353 LYS) of the target by forming chemical bond will hinder the function of the target Angiotensin-converting enzyme 2 (ACE2) receptors - PDB- 2AJF being recognized as binding site for novel corona virus for its pathogenesis essential for host-viral interaction. Thereby phytocomponents which inhibit the target ACE-2 may act as a possible therapeutic agent for management of COVID-19 and related symptoms.

3D- Structure of Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Crystalline structure of the target protein Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF was retrieved from protein data bank and protein clean-up process was done and essential missing atom were being added. Different orientation of the lead molecules with reference to the target protein was evaluated by Autodock program and also the best dock pose was selected supported based on the interaction study analysis

METHODOLOGY

Docking calculations were dispensed for retrieved phytocomponents against target protein ACE-2. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the help of AutoDock tools (*Morris, Goodsell et al., 1998*). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (*Morris, Goodsell et al., 1998*). AutoDock parameter set- and distance-dependent dielectric functions were employed in the calculation of the van der Waals and also the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (*Solis and Wets, 1981*). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

OBSERVATION AND INFERENCE

Total of 4 bioactive lead compounds were retrieved from the herbs present in the formulations. From reported data of the herb, the lead molecule Lupeol possess 100% binding efficacy by interacting with both the core target amino acids (31 LYS and 353 LYS) present on the target. Followed by this other phytocompounds such as Angelicin and Umbelliferone possess 50% affinity by binding with one of the target amino acid either with 31 LYS or with 353 LYS present on the target receptor ACE-2.

CONCLUSION

Based on the results of the computational analysis it was concluded that the bio-active compound Lupeol present in the formulations revels significant binding against the target protein thereby it was concluded that these compounds may exerts promising inhibiting against ACE-2 receptor and hereby halt the host-viral interface.

REFERENCES

1. Bikadi, Z., Hazai, E.*Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock*. J. Cheminf. 1, 15 (2009)
2. T. A. Halgren. *Merck molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94*. Journal of Computational Chemistry 17 (5-6), 490-519 (1998)
3. G. M. Morris, D. S. Goodsell, et al. *Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function*. Journal of Computational Chemistry 19 (14), 1639-1662(1998)
4. F. J. Solis and R. J. B. Wets. *Minimization by Random Search Techniques*
5. Kiran G., Karthik L., Devi M.S., Sathiyarajeswaran P., Kanakavalli K., Kumar K.M., Kumar D.R. In silico computational screening of Kabasura Kudineer-official Siddha formulation and JACOM against SARS-CoV-2 spike protein. *Journal of Ayurveda and integrative medicine.*
6. Anshul Chawla., Ramandeep Kaur., Anilkumar Sharma. *Ficus carica Linn.. A review on its pharmacognostic, phytochemical and pharmacological aspects,* Int.J.Pharm.Phytopharmacol.Res 2012, 1 (4) : 215-232.

Table 1. List of Phytocomponents Selected for docking

S.N	Name of the Herb	Phyto components	References
1.	*Ficus carica*	• Menthol	AIrena V Lightbourn.Crude Edible Fig (Ficus carica) Leaf Extract Prevents Diethylstilbestrol (DES)-Induced DNA Strand Breaks in Single-Cell Gel Electrophoresis (SCGE)/Comet Assay: Literature Review and Pilot Study.J Bioequivalence Bioavailab. 2019; 11(2): 19–28.
2.	*Ficus carica*	• Angelicin • Umbelliferone	Shukranul Mawa.Ficus carica L. (Moraceae): Phytochemistry, Traditional Uses and Biological Activities.Evid Based Complement Alternat Med. 2013; 2013: 974256.
3.	*Ficus carica*	• Lupeol	Ghadam Ali Khodarahmi.Cytotoxic Effects of Various Extracts and Latex of Ficus carica L. on HeLa cell Line.Iran J Pharm Res. 2011 Spring; 10(2): 273–277.

Table 2. Receptor

PDB	Name of the Target
2AJF	Angiotensin-converting enzyme 2 (ACE2) receptor

Table 3. Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Angelicin	186.16 g/mol	$C_{11}H_6O_3$	0	3	0
Umbelliferone	162.14g/mol	$C_9H_6O_3$	1	3	0
Lupeol	426.7g/mol	$C_{30}H_{50}O$	1	1	1

Table 4. Summary of the molecular docking studies of compounds against Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Menthol	-5.11	180.63	-0.07	-5.70	457.47
Angelicin	-3.25	4.17*	-0.02	-3.25	313.99
Umbelliferone	-3.15	4.90*	-0.24	-3.45	294.89
Lupeol	-5.24	144.70	-0.05	-6.10	564.37

Table 5. Amino acid Residue Interaction of Lead against Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Molecule	Interactions	Amino Acid Residue- Binding					
Menthol	0	37 GLU	40 PHE	350 ASP	390 PHE	393 ARG	
Angelicin	1	31 LYS	34 HIS	35 GLU			
Lupeol	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS
Umbelliferone	1	31 LYS	34 HIS	35 GLU			

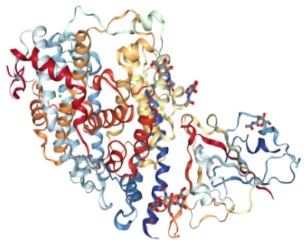


Fig.1. Receptor structure

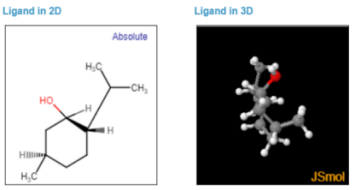


Fig.2. Menthol

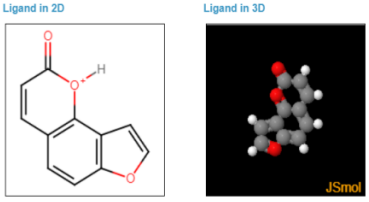


Fig.3. Angelicin

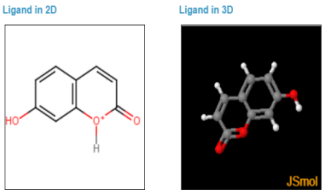


Fig.4. Umbelliferone

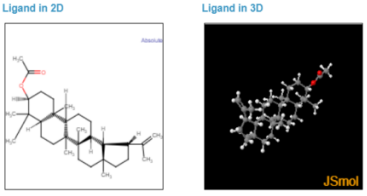


Fig.5. Lupeol

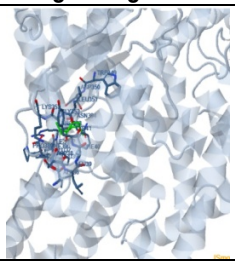
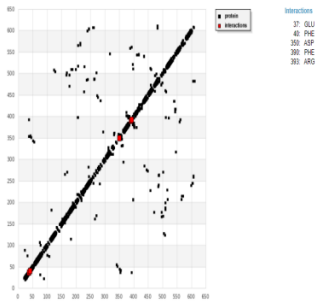
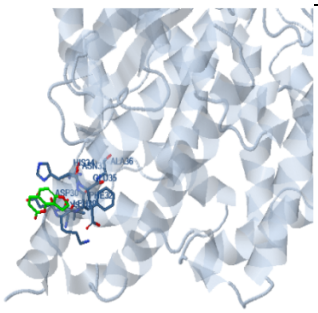


Fig.6. Menthol with angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

		
Fig.7. 2d interaction plot	**Fig.8. Hydrogen bond plotting with core amino acid analysis**	**Fig.9. Angelicin with angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF**

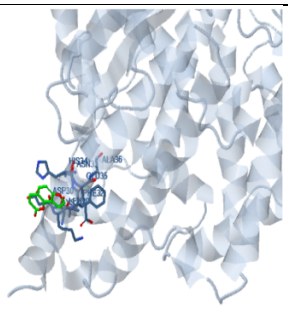
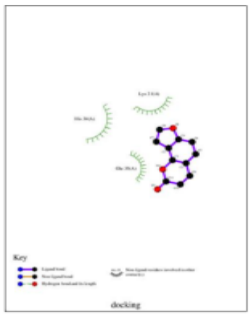
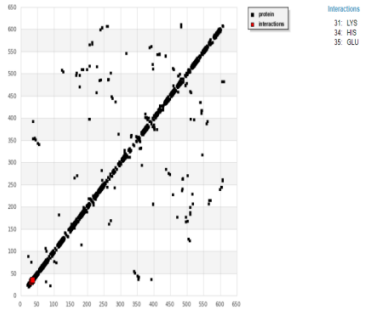

		

Fig.10. 2d interaction plot	**Fig.11. Hydrogen bond plotting with core amino acid analysis**	**Fig.12. Umbelliferone with angiotensin- converting enzyme 2 (ACE2) receptor- PDB 2AJF**

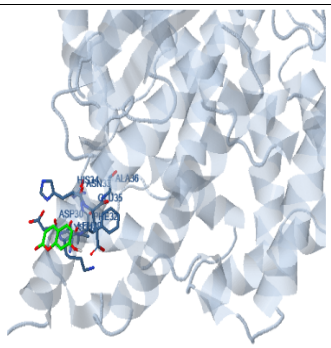
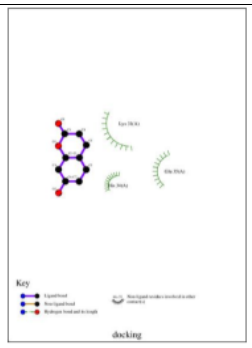
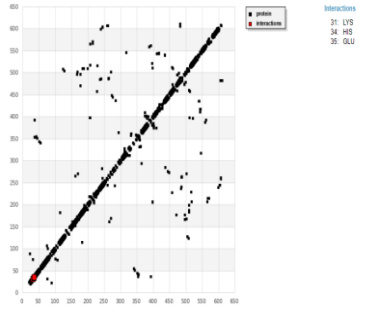

		

Fig.13. 2d interaction plot	**Fig.14. Hydrogen bond plotting with core amino acid analysis**	**Fig.15. Lupeol with angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF**

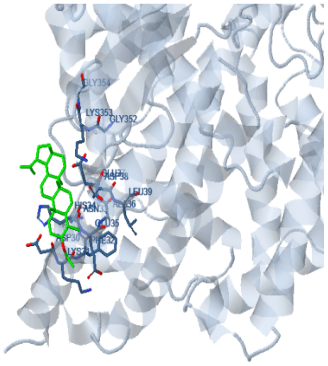


Fig.16. 2d interaction plot

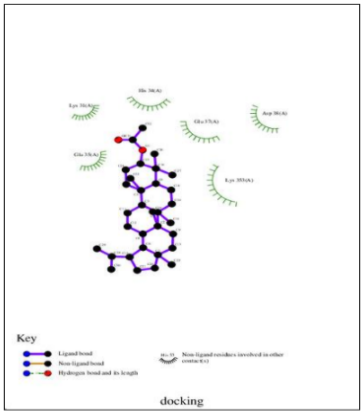


Fig.17. Hydrogen bond plotting with core amino acid analysis

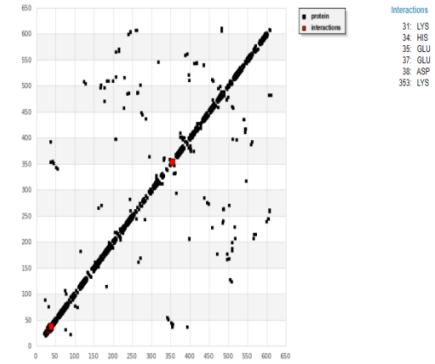


Fig.18. converting enzyme 2 (ACE2) receptor- PDB 2AJF

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RESEARCH ARTICLE

Acute and Subacute Toxicity Studies of Putru Pathangam, A Siddha Herbo Mineral Formulation in Rats

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ABSTRACT

Researchers say cancer treatment will gradually improve and perhaps in another thirty years we may boast something that works for several types of cancers. In view of the fact that cancer panacea is long way off according to modern scientists, it is high time we have to explore the remedies described in Siddha system of Medicine. Many formulations has been mentioned in various Siddha classical texts. Putru pathangam is one among them indicated for putru noi (cancer)To explore the effectiveness and safety of Siddha medicine to scientific world, Putur pathangam has been subjected to acute and sub acute toxicity study to prove the safety of the medicine and this study proved the safety of the medicine Putru pathangam, which is indicated for putru noi.

key words; Putru pathangam, putrunoi, Siddha, cancer, toxicity

INTRODUCTION

Cancer, one of the leading cause of death worldwide (accounting for 8.2 million deaths in 2012), continues to affect people at an alarmingly increasing rate globally and it is projected by the WHO that cancer burden would increase to 20 million by 2020 with 70% in the developing world. In the developing countries, certain infections are the main reasons for 20% of the cancer death. According to Siddha, anyone or two or all of the three *thodangal,* namely *Vali, Azhal and Iyam,* may get vitiated due to a many factors including improper food habbit and sedentary lifestyle. Chewing tobacco, Smoking, inhaling in chemical fumes or eating foods and fruits that have been heavily processed with chemicals during cultivation also aggravate a *thodam* in the body. The blood along with the aggravated *thodam* circulates in the whole body and relocates in a region that has week immunity. As a result, the normal function of blood in that region is disrupted. One of main the functions of blood is transporting *Pranan* (life air) to each cell, the smallest functional unit in the body. *Pranan*, according to Siddha, is not only oxygen but also contains a subtle force or energy, which empower the cell to perform its normal functions including its division. Due to localization of improper *Raththa thathu*, the *Pranan* gets to the particular site of the cells also becomes impure. This lead to improper excess division of cells, it is termed as cancer. If this is not detected at an early phase and proper measures are not taken, the impurity spreads to other regions having week immunity. Thus the cancer spreads to other organs of the body.

Over the years scientists have been trying hard to find out a cure for cancer, which is incurable and fatal unless identified early on. Researchers say cancer treatment will gradually improve and perhaps in another thirty years we may boast something that works for several types of cancers. In view of the fact that cancer panacea is long way off according to modern scientists, it is high time we have to explore the remedies described in Siddha system of Medicine. Formulations, mentioned in Siddha System of Medicine, consisting of multiple herbs and minerals possess tremendous potential for a cancer cure. Siddha anti cancerous herbs are reported to work on multiple biochemical pathways and are capable of influencing a number of organ systems simultaneously. The benefits of this formulation apart from being a potential anti-cancer drug as per the literature, it is also expected to nourish the body as a whole by supporting various organ systems and to be easily available.The present study is to explore the safety of *Putru Pathangam* (PP), a Siddha herbo-mineral medicine mentioned in the text *Anuboga Vaithiya Navaneetham* – Part 10 through animal model

METHODS

Acute and subacute oral toxicity has been carried out with approval IEC, National Institute of Siddha

Animal husbandary and preparation

The temperature maintained in animal experimental room was 22ºC (+3ºC)., relative humidity was at least 30% . Lighting was artificial, the sequence being 12 hrs light, 12 hrs dark. For feeding, conventional laboratory diets was used and limitless of drinking water was supplied. Animals was grouped and tagged by dose, but the number of animals per cage not interfered with clear observations of each animal. The animals were randomly selected and marked for the purpose of individual identification, then kept in their cages for 7 days prior to study for acclimatization to the laboratory conditions.

Acute Oral Toxicity

Acute toxicity study was carried out as per OECD-423 guideline. Healthy Wistar albino female rat weighing between 200gm and 220 gm. Study carried out at three female rats under fasting condition. Observed every one hour for signs of toxicity for first 24 hours, then daily for about 14 days. The preferred rodent species was healthy young adult strain Wistar female albino rat, age of the animal between 8 and 12 weeks old and its weight fell in an interval within ±20 % of the mean weight of the animals, were used which should be nulliparous and non-pregnant.

Observation done

In the acute toxicity study, the female albino mice were treated with different concentration of Putru pathangam from the range of 5mg/kg to 100mg/kg which did not produce signs of behavioural changes, toxicity, and mortality in animals and observed during 14 days of the experimental period. The acute toxicity study also revealed that the drug was found to be safe upto 100 mg/kg bw. Body weight change in drug treated animals was found normal. In this study Putru pathangam was found to be safe up to 100 mg/kg, p.o.

SUB ACUTE TOXICITY STUDIES (28 DAYS)

28-days repeated oral toxicity study was performed according to OECD test guideline 407 - Repeated Dose 28-Days Oral Toxicity Study in Rodents. Forty young healthy adult Wistar albino rats weighing between 100-120 gm/ b.wt., were used for the study. Every Animal was kept separate with a well ventilated polypropylene. 12-h light/12-h dark artificial photoperiod was maintained. Room temperature 22°C (±3°C) and relative humidity 50–70% were maintained in the room. Animals had free access to pelleted feed and Reverse osmosis (Rios, USA) purified water *ad libitum*. As mentioned in guidliness, Selected albino rats were acclimatized for 7 days to the laboratory environment prior to start of the study and randomized into four groups (10 animals/group; 5/sex) based on stratified body weight method.

Experiment details

After the acclimatized period, all the animals were grouped randomly distributed into control group and drug treated group for three different doses like low dose(12 mg/bw/d), mid dose(24 mg/bw/d) and high dose(48 mg/bw/d). The animals were administrated with the trail drug once daily for 28 days. The animals in normal control group received normal saline 5 ml/kg b.w. The animals in group 2,3,4 received the trail drug Putru pathangam with low dose(12mg/bw/d), Mid dose (24mg/bw/d) and High dose (48 mg/bw/d).

Observation

- Body weight was recorded once in a week till completion of the experiment.
- Animals were observed for mortality twice daily till the completion of experiment.
- Following test drug administration, experimental animals were observed daily for clinical signs till completion of the experiment.
- Feed consumption of individual animals was recorded daily till completion of the experiment.

Blood Parameters

Blood samples were collected through retro orbital puncture on day 29. Prior to blood collection, the animals were overnight fasted but had free access to water. Blood samples were taken from experimental animal to analyse following parameters.

Haematology

Haemoglobin, Hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), Total erythrocyte count (RBC), mean corpuscular hemoglobin concentration (MCHC), platelet count and total leucocytes (WBC).

Biochemistry

- Carbohydrate mechanism: Glucose
- Lipid metabolism : Total cholesterol, triglycerides
- Protein metabolism : Total protein, albumin
- Liver function Hepatocellular : Glutamyl pyruvate aminotransferase (GPT), Hepatobiliary : Alkaline phosphatase, γ-glutamyl transferase, Total bilirubin, bile acid

Renal function : Creatinine and urea

Necropsy

All survived animals were sacrificed using CO_2 euthanasia on day 29. The following were the observations carried out during necropsy.

Gross Pathology

All the experimental animals were subjected to detailed gross necropsy which includes gross examination of external orifices, skin with mammary gland, thymus, lymph nodes, eyes, brain, trachea, thyroid, heart, lungs, stomach, small and large intestines (with peyer's patches), spleen, liver, adrenals, kidneys, urinary bladder, testes, epididymides, male sex glands (as whole), ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone with bone marrow, spinal cord and other gross lesions.

Relative Organ Weight

Absolute weight of brain, heart, liver, paired kidneys, paired adrenals, spleen, paired testes, paired epididymides, male sex glands, uterus with cervix, paired ovaries and thymus was recorded at necropsy. Absolute organ weight was converted into relative organ weight and expressed in percentage as mentioned below

$$\text{Relative organ weight (\%)} = \frac{\text{Weight of the organ (g) X 100}}{\text{Final body weight (g) of animal}}$$

Histopathology - Histopathology examination was performed for below mentioned organs of animals from control and high dose groups and for the organs from low and mid dose groups that showed no evidence of gross abnormalities. Organs such as skin with mammary gland, lymph nodes, eyes, brain, trachea, thyroid, thymus, heart, lungs, stomach, small and large intestines (with peyer's patches), spleen, liver, adrenals, kidneys, urinary bladder, testes, epididymides, male sex glands (as whole), ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone with bone marrow and spinal cord of all the animals were taken and they were fixed in 10% Neutral buffered formalin for 48 hrs, processed for paraffin embedment, sectioned and stained with H&E for histopathological evaluation.

RESULT AND DISCUSSION

In Subacute toxicity study, there were no treatment related deaths, abnormal clinical signs, remarkable body weight changes or differences in feed consumption were observed in test drug administrated rats . No significant difference in hematological parameters such as HCT, HGB, RBC, MCH, MCV, MCHC, platelet count and WBC were observed between the control and test drug administered animals. No significant difference in glucose, total cholesterol, triglycerides, total protein, albumin, glutamyl pyruvate aminotransferase (GPT), alkaline phosphatase, γ-glutamyl transferase, total bilirubin creatinine and urea were observed between the control and test drug administered animals. No significant difference in any of the organs weight was observed between the control and test drug administered animals. No gross and histopathological findings were observed in all the experimental animals. NOAEL of Putru pathangam was found to be > 24mg/kg when administered for a period of 28 days in rats. No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days. The increase or decrease in the values obtained was within normal biological limits or the effect was not dose dependent.

Effect of PUTRU PATHANGAM on body weight changes – sub acute study report (Table.1)

After administration of Putru pathangam for different groups (control, low dose, mid dose and high dose) the change in body weight on every seventh day for subsequent 28 days(0,7th ,14th ,21st and 28th) were recorded for Male and Female. The observed data for Male(M) and Female (F) and their average (MF) and presented here

Effect of PUTRU PATHANGAM on feed consumption -sub acute study report (Table.2)

The feed consumption for different groups (control, low dose, mid dose and high dose) were recorded for Male and Female on every week end for four weeks subsequently (I,II,III and IV) , after administration of Putru pathangam . The observed data of feed consumption of Male(M) , Female (F) and their average (MF) are presented here

Effect of Putru pathangam on haematology (Table.3)

The WBC, RBC, HGB, HCT, MCV, MCH,MCHC and PLT values for male and female rats with control , low dose , mid dose and high dose of Putru Pathangam were observed . The different parameters for Male(M),Female (F) and their average are presented here.

Effect of PUTRU PATHANGAM on Plasma biochemistry – sub acute study report (Table.4)

The values of Gucose, TGL, total Cholesterol, SGPT, ALP, y-GT , BUN, LDH, T.Protein, Albumin, Creatinine for male and female rats after administration of control, low dose , Mid dose and High dose of Putru pathangam were analysed from their blood samples. The values are given below.

CONCLUSION

Based on the acute and 28 days repeated oral toxicity Putru , No Observed Adverse Effect Level (NOAEL) of Putru pathangam, A Siddha herbo mineral formulations in the maximum dose of 48mg/kg/body weight of animal. This study substantiates the safety of medicine Putru pathangam in the treatment of cancer and also safety of traditional system of medicine. This will be pave the way for further elaborated pharmacological and subsequent clinical study of this drug on cancer

REFERENCES

1. http://www.who.int/mediacentre/factsheets/fs297/en/
2. National Cancer Registry Programme (NCRP, ICMR). Consolidated report of population based cancer registries 2004-2005. Bangalore: NCRP; 2008.
3. Indian J Med Res 130, September 2009, pp 219-221
4. Sahibu PMA. Anuboga vaithya navaneethan-Part 10(Tamil). Chennai: Tamarai noolagam: 1995. P 53
5. Chang, M.C., et. al, J.H., 2001. Areca nut extract and arecoline induced the cell cycle arrest but not apoptosis of cultured oral KB epithelial cells: association of glutathione, reactive oxygen species and mitochondrial membrane potential. Carcinogenesis. 22: 1527–1535.
6. Dr.R.Thiyagarajan, Gunapadam Part II (Thathu Jeeva vaguppu), page no-283, Edition-4th, 2004.
7. Ramaswamy et al.: Acute toxicity and the 28-day repeated dose study of a Siddha medicine Nuna Kadugu in rats. BMC Complementary and Alternative Medicine 2012 12:190.

Table 1. Effect of Putru pathangam on body weight changes

Group	Treatment	Sex	Body weight (kg)				
			Day 0	Day 7	Day 14	Day 21	Day 28
I	Control	M	140.20±4.51	148.80±5.42	162.00±5.10	169.00±5.31	177.60±4.47
		F	132.80±4.27	142.60±3.93	149.40±4.61	157.00±4.71	169.80±4.97
		MF	136.50±3.18	145.70±3.32	155.70±3.86	163.00±3.90	173.70±3.41
II	Low dose	M	140.40±5.21	149.40±6.42	159.60±5.49	168.20±5.81	176.80±5.21
		F	130.80±5.70	139.60±5.24	149.00±4.47	158.80±5.24	169.40±4.92
		MF	135.60±3.98	144.50±4.23	154.30±3.78	163.50±4.01	173.10±3.59
III	Mid dose	M	142.40±4.30	149.60±4.23	158.40±4.23	165.00±4.32	175.40±3.17

		F	133.60±6.35	142.40±5.69	151.60±5.47	160.20±5.45	168.80±5.21
		MF	138.00±3.90	146.00±3.55	155.00±3.45	162.60±3.38	172.10±3.08
IV	High dose	M	140.40±4.01	149.80±3.28	159.40±4.34	167.60±3.34	173.60±3.54
		F	132.40±3.91	139.80±5.05	151.00±3.94	159.80±5.28	166.40±5.33
		MF	136.40±2.96	144.80±3.29	155.20±3.10	163.70±3.22	170.00±3.25

Values expressed in mean ± SEM

Table 2. Effect of Putru pathangam on feed consumption

Group	Treatment	Sex	Cumulative feed intake (kg)			
			I Week	II Week	III Week	IV Week
I	Control	M	53.43±9.42	53.43±2.47	53.43±4.35	66.71±4.32
		F	47.57±6.41	54.57±3.19	59.14±1.94	55.14±4.18
		MF	50.50±7.92	54.00±2.83	56.29±3.15	60.93±4.25
II	Low dose	M	66.86±5.38	65.43±6.88	62.57±3.86	62.71±4.07
		F	60.29±4.82	61.29±2.56	63.43±3.53	65.71±4.92
		MF	63.57±5.10	63.36±4.72	63.00±3.70	64.21±4.50
III	Mid dose	M	53.00±5.37	52.29±5.85	59.43±4.32	60.86±5.22
		F	61.00±3.69	54.71±3.00	66.14±3.81	66.57±3.01
		MF	57.00±4.53	53.50±4.42	62.79±4.02	63.71±4.12
IV	High dose	M	66.00±3.56	56.86±4.07	51.29±3.02	59.00±1.41
		F	54.86±3.71	60.29±1.46	60.14±3.99	64.29±1.67
		MF	60.43±3.63	58.57±2.76	55.71±3.51	61.54±1.54

Values expressed in mean ± SEM

Table.3 Effect of Putru pathangam on haematology

Group	Treatment	Sex	WBC (10^3/uL)	RBC (10^6/uL)	HGB (%)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3/µL)
I	Control	M	8.52±0.80	6.93±0.36	15.10±0.25	52.40±2.21	71.40±0.30	20.76±1.03	29.08±1.49	1639.80±104.28
		F	6.60±1.13	6.68±0.32	14.06±0.54	47.62±2.17	70.98±0.22	20.70±0.55	30.14±0.73	1307.20±73.63
		MF	7.56±0.73	6.80±0.23	14.58±0.33	50.01±1.66	71.19±0.19	20.73±0.55	29.61±0.80	1473.50±81.82
II	Low dose	M	7.84±0.87	6.36±0.29	14.58±0.42	45.70±2.24	71.76±0.22	22.58±0.32	32.06±0.65	1801.80±40.94
		F	6.08±0.50	6.36±0.32	13.50±0.84	45.58±2.32	71.70±0.18	21.18±0.50	29.52±0.65	1393.00±36.27
		MF	6.96±0.56	6.36±0.20	14. 04±0.48	45.64±1.52	71.73±0.14	21.88±0.36	30.79±0.61	1597.40±72.85
III	Mid dose	M	7.20±0.91	6.37±0.28	14.16±0.24	45.94±1.85	72.36±1.55	22.36±0.70	30.96±0.86	1638.20±100.99
		F	6.38±0.48	6.35±0.38	13.60±0.78	45.24±2.84	71.02±0.35	21.46±0.41	30.12±0.59	1457.80±35.67
		MF	6.79±0.50	6.36±0.22	13.88±0.39	45.59±1.60	71.69±0.78	21.91±0.41	30.54±0.51	1548.00±58.76
IV	High dose	M	7.44±0.43	5.91±0.24	13.56±0.54	42.02±1.81	71.02±0.39	22.92±0.41	32.30±0.74	1649.60±89.26
		F	6.02±0.56	6.58±0.16	14.44±0.15	46.66±1.17	70.88±0.10	21.50±0.46	31.02±0.49	1497.40±58.17
		MF	6.73±0.41	6.25±0.18	14.00±0.30	44.34±1.28	70.95±0.19	22.21±0.37	31.66±0.47	1573.50±56.27

Values expressed in mean ± SEM

Table.4 Effect of PUTRU PATHANGAM on Plasma biochemistry – sub acute study report

Group	Treatment	Sex	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	SGPT (U/L)	ALP (U/L)	γ-GT (U/L)
I	Control	M	94.94±11.27	57.55±7.93	47.91±5.55	69.87±4.57	215.10±15.68	5.81±0.85
		F	102.81±10.14	55.70±6.43	55.98±8.18	51.01±4.95	299.62±34.32	7.62±1.78
		MF	98.88±7.27	56.62±4.82	51.94±4.85	60.44±4.47	257.36±22.69	6.71±0.98
II	Low dose	M	105.73±10.25	54.44±7.29	52.20±6.46	52.22±3.39	290.80±19.68	7.32±1.90
		F	112.71±3.86	80.50±5.58	62.52±4.47	68.92±7.92	282.23±19.19	6.30±0.39
		MF	109.22±5.29	67.47±6.13	57.36±4.08	60.57±4.92	286.52±13.04	6.81±0.93
III	Mid dose	M	85.68±4.82	46.69±3.61	50.31±1.65	68.28±9.15	198.91±19.70	4.85±0.57
		F	96.95±2.65	49.61±1.11	49.32±3.43	52.10±2.10	182.05±38.46	4.88±0.75
		MF	91.32±3.20	48.15±1.85	49.81±1.80	60.19±5.18	190.48±20.56	4.86±0.44
IV	High dose	M	86.74±4.12	66.26±10.31	48.05±3.53	42.12±2.24	219.34±32.94	4.05±1.65
		F	85.48±2.44	83.71±9.51	48.05±2.27	57.17±6.42	307.23±38.39	6.55±0.84
		MF	86.11±2.27	74.99±7.22	48.05±1.98	49.65±4.07	263.28±27.99	5.30±0.97

Values expressed in mean ± SEM

(continuation of table 4)

Group	Treatment	Sex	BUN (mg/dL)	LDH (U/L)	T. Protein (g/dL)	Albumin (g/dL)	Creatinine (mg/dL)
I	Control	M	12.60±0.60	426.50±28.59	4.16±0.18	1.49±0.09	0.33±0.04
		F	11.94±0.75	475.28±51.05	4.59±0.39	1.98±0.19	0.39±0.03
		MF	12.27±0.47	450.89±28.75	4.38±0.22	1.74±0.13	0.36±0.02
II	Low dose	M	11.69±0.92	557.80±72.08	4.10±0.25	1.75±0.31	0.32±0.04
		F	12.60±0.38	492.62±40.29	4.93±0.14	1.79±0.26	0.30±0.02
		MF	12.15±0.49	525.21±40.41	4.52±0.19	1.77±0.19	0.31±0.02
III	Mid dose	M	13.43±0.85	431.41±71.12	4.17±0.16	1.69±0.15	0.35±0.03
		F	11.12±0.43	325.03±69.67	4.35±0.13	1.81±0.09	0.53±0.09
		MF	12.27±0.59	378.22±50.17	4.26±0.10	1.75±0.09	0.44±0.05
IV	High dose	M	12.09±1.29	378.98±57.15	4.59±0.52	1.68±0.07	0.45±0.06
		F	11.91±0.86	396.76±48.33	3.93±0.19	1.83±0.08	0.43±0.05
		MF	12.00±0.73	387.87±35.41	4.26±0.28	1.76±0.06	0.44±0.03

Values expressed in mean ± SEM

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RESEARCH ARTICLE

Some Properties of Operations on αg-open sets

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ABSTRACT

The objective of this paper is to introduce new notions namely αg_γ-interior, αg-interior$_\gamma$, αg_γ-kernel and αg_γ-g.closed sets by using γ-operation on $\tau_{\alpha g}$ and analyze their properties in topological spaces.

Keywords: topological space, αg_γ-interior, αg-interior$_\gamma$, αg_γ-kernel and αg_γ-g.closed sets.

INTRODUCTION

The concept of α-open sets was introduced by Njastad [7] in 1965. Levine [4] initiated the study of generalized closed sets in the year 1970. Maki et al. [5] introduced αg-closed sets in the year 1994. The concept of an operation on topological spaces was initiated by Kasahara [3] and also he brought forth the notion of α-closed graphs of functions in topological spaces. Jankovic [2] analyzed the functions with α-closed graphs. Later, Ogata [8] renamed the operation α as γ-operation and studied γ-open sets in topological spaces. Mershia Rabuni and Balamani [6] defined the operation γ on $\tau_{\alpha g}$ and introduced αg_γ-open sets in topological spaces. In the present paper, we carry on with the investigation on some of the topological properties of closure, interior and kernel operators. Also, we define a new class of generalized set called αg_γ-g.closed set by using the operation γ on $\tau_{\alpha g}$ and analyze some of its properties in topological spaces.

Preliminaries

Throughout this paper (X, τ) signifies a topological space on which no separation axiom is assumed unless otherwise mentioned and γ denotes an operation on $\tau_{\alpha g}$. For any "subset A of a space (X, τ), $cl(A)$ and $int(A)$ denote the closure and interior of A respectively.

Definition 2.1 Let (X,τ) be a topological space. An operation γ on $\tau_{\alpha g}$ is a mapping from $\tau_{\alpha g}$ into the power set $P(X)$ of X ∋ $V \subseteq \gamma(V)$ ∀ $V \in \tau_{\alpha g}$, the value of V under the operation γ is denoted by $\gamma(V)$.

Definition 2.2 A non-empty subset A of (X,τ) with an operation γ on $\tau_{\alpha g}$ is called an αg_γ-open set if ∀ $x \in A$, ∃ an αg-open set U ∋ $x \in U$ and $\gamma(U) \subseteq A$. The collection of all αg_γ-open sets in (X,τ) is denoted by $\tau_{\alpha g_\gamma}$. The complement of an αg_γ-open set is called αg_γ-closed.

Definition 2.3 An operation $\gamma : \tau_{\alpha g} \to P(X)$ is called αg-regular if ∀ $x \in X$ and ∀ pair of αg-open sets A and B containing x, ∃ an αg-open set C containing x ∋ $\gamma(A) \cap \gamma(B) \supseteq \gamma(C)$.

Definition 2.4 An operation γ on $\tau_{\alpha g}$ is said to be αg-open if ∀ αg-open set U containing $x \in X$, ∃ an αg_γ-open set V ∋ $x \in V$ and $V \subseteq \gamma(U)$.

Definition 2.5 Let γ be an operation on $\tau_{\alpha g}$. A point $x \in X$ is said to be an αg_γ-closure point of a set A if $\gamma(U) \cap A \neq \emptyset$ ∀ αg-open set U containing x.
$\alpha g\ Cl_\gamma(A) = \{x \in X : \gamma(U) \cap A \neq \emptyset, \forall\ \alpha g\text{-open set } U \text{ containing } x\}$.

Definition 2.6 Let γ be an operation on $\tau_{\alpha g}$. Then $\alpha g_\gamma Cl(A)$ is defined as the intersection of all αg_γ-closed sets containing A.
$\alpha g_\gamma Cl(A) = \cap \{F \subseteq X : A \subseteq F \text{ where } X \backslash F \in \tau_{\alpha g_\gamma}\}$.

Some Properties of Operations on αg-open sets

Definition 3.1 Let A be a subset of a topological space (X,τ) and γ be an operation on $\tau_{\alpha g}$. A point $x \in A$ is said to be an αg_γ-interior point of A if ∃ an αg-open set V of X containing x ∋ $\gamma(V) \subseteq A$." $\alpha g int_\gamma(A)$ denotes the set of all such αg_γ-interior points of A.
Thus, $\alpha g int_\gamma(A) = \{x \in A : x \in V \in \tau_{\alpha g} \text{ and } \gamma(V) \subseteq A\}$

Proposition 3.2 Let A and B be subsets of X. Then
(i) $\alpha g int_\gamma(A) \subseteq A$
(ii) A is αg_γ-open iff $A = \alpha g int_\gamma(A)$.
(iii) If $A \subseteq B$, then $\alpha g int_\gamma(A) \subseteq \alpha g int_\gamma(B)$
(iv) $\alpha g int_\gamma(A) \cup \alpha g int_\gamma(B) \subseteq \alpha g int_\gamma(A \cup B)$
(v) $\alpha g int_\gamma(A \cap B) \subseteq \alpha g int_\gamma(A) \cap \alpha g int_\gamma(B)$
(vi) If γ is αg-regular, then $\alpha g int_\gamma(A \cap B) = \alpha g int_\gamma(A) \cap \alpha g int_\gamma(B)$
(vii) $\alpha g int_\gamma\left(\alpha g int_\gamma(A)\right) \subseteq \alpha g int_\gamma(A)$

Proof:
(i) (iii) (iv) & (v) Obvious.
(ii) If $A = \alpha g int_\gamma(A)$, then by Definition 3.1, ∀ $x \in \alpha g int_\gamma(A)$, ∃ an αg-open set V of X containing x ∋ $\gamma(V) \subseteq A$.

Therefore, A is αg_γ-open. Conversely, consider A to be an αg_γ-open set and $x \in A$. Since A is αg_γ-open, ∀ $x \in A$, ∃ an αg-open set U ∋ $x \in U$ and $\gamma(U) \subseteq A$ which implies that x is an αg_γ-interior point of A. i.e., $x \in \alpha g int_\gamma(A)$.
Therefore $A \subseteq \alpha g int_\gamma(A)$. By (i), $\alpha g int_\gamma(A) \subseteq A$. Hence $A = \alpha g int_\gamma(A)$.
(vi) By (v), $\alpha g int_\gamma(A \cap B) \subseteq \alpha g int_\gamma(A) \cap \alpha g int_\gamma(B)$. Let $x \in \alpha g int_\gamma(A) \cap \alpha g int_\gamma(B)$ which implies that $x \in \alpha g int_\gamma(A)$ and $x \in \alpha g int_\gamma(B)$. Then by Definition 3.1, ∃ an αg-open sets U and V containing x ∋ $\gamma(U) \subseteq A$ and $\gamma(V) \subseteq B$, then $\gamma(U) \cap \gamma(V) \subseteq A \cap B$. Since γ is αg-regular, ∃ an αg-open set W containing x ∋ $\gamma(W) \subseteq \gamma(U) \cap \gamma(V)$. i.e., $\gamma(W) \subseteq$

$\gamma(U) \cap \gamma(V) \subseteq A \cap B$ which implies that $x \in \alpha gint_\gamma(A \cap B)$. Therefore, $\alpha gint_\gamma(A) \cap \alpha gint_\gamma(B) \subseteq \alpha gint_\gamma(A \cap B)$. Hence we have the equality.
(vii) Follows from (i) and (iii).

Remark 3.3 The reverse inclusion of (vii) in Proposition 3.2 need not be true.
Example 3.4 Let $X = \{a, b, c\}$ and $\tau = \{\emptyset, \{a\}, \{b\}, \{a, b\}, \{a, c\}, X\}$. Then $\tau_{\alpha g} = \{\emptyset, \{a\}, \{b\}, \{a, b\}, \{a, c\}, X\}$. Let $\gamma: \tau_{\alpha g} \to P(X)$ be an operation on $\tau_{\alpha g}$ defined by

$$\gamma(A) = \begin{cases} cl(A) & if\ A\ is\ singleton \\ X & otherwise \end{cases} \quad \forall\ A \in \tau_{\alpha g}$$

Then $\tau_{\alpha g_\gamma} = \{\emptyset, \{b\}, X\}$. Here for $A = \{a, c\}$, $\alpha gint_\gamma(A) = \{a\}$ whereas $\alpha gint_\gamma\left(\alpha gint_\gamma(A)\right) = \emptyset$. Therefore, $\alpha gint_\gamma(A) \nsubseteq \alpha gint_\gamma\left(\alpha gint_\gamma(A)\right)$.

Remark 3.5 $\alpha gint_\gamma(A)$ need not be αg-open.
Example 3.6 Let $X = \{a, b, c\}$ and $\tau = \{\emptyset, \{a\}, X\}$. Then $\tau_{\alpha g} = P(X) \backslash \{b, c\}$. Let $\gamma: \tau_{\alpha g} \to P(X)$ be an operation on $\tau_{\alpha g}$ defined by

$$\gamma(A) = \begin{cases} A & if\ a \in A \\ cl(A) & otherwise \end{cases} \quad \forall\ A \in \tau_{\alpha g}$$

Then $\tau_{\alpha g_\gamma} = \{\emptyset, \{a\}, \{a, b\}, \{b, c\}, \{a, c\}, X\}$. Here for $A = \{b, c\}$, $\alpha gint_\gamma(A) = \{b, c\}$ which is not αg-open in (X, τ).
Remark 3.7 $\alpha gint_\gamma(A)$ need not be αg_γ-open.
Example 3.8 Let $X = \{1,2,3\}$ and $\tau = \{\emptyset, \{1\}, \{2\}, \{1,2\}, X\}$. Then $\tau_{\alpha g} = \{\emptyset, \{1\}, \{2\}, \{1,2\}, X\}$. Let $\gamma: \tau_{\alpha g} \to P(X)$ be an operation on $\tau_{\alpha g}$ defined by

$$\gamma(A) = \begin{cases} A & if\ e \in A \\ A \cup \{3\} & otherwise \end{cases} \quad \forall\ A \in \tau_{\alpha g}$$

Then $\tau_{\alpha g_\gamma} = \{\emptyset, \{1\}, \{1,2\}, X\}$. Here for $A = \{2,3\}$, $\alpha gint_\gamma(A) = \{2\}$ which is not αg_γ-open in (X, τ).

Theorem 3.9 For any subset G of X,
(i) $\alpha gint_\gamma(X \backslash G) = X \backslash \alpha gcl_\gamma(G)$
(ii) $\alpha gcl_\gamma(X \backslash G) = X \backslash \alpha gint_\gamma(G)$
(iii) $\alpha gint_\gamma(G) = X \backslash \alpha gcl_\gamma(X \backslash G)$
(iv) $\alpha gcl_\gamma(G) = X \backslash \alpha gint_\gamma(X \backslash G)$

Proof: (i) Let $x \in \alpha gint_\gamma(X \backslash G)$**,** then $\exists$ an αg-open set V of X containing $x \ni \gamma(V) \subseteq X \backslash G$. Then $\gamma(V) \cap G = \phi$ which implies that $x \notin \alpha gcl_\gamma(G)$. Therefore, $x \in X \backslash \alpha gcl_\gamma(G)$. Hence, $\alpha gint_\gamma(X \backslash G) \subseteq X \backslash \alpha gcl_\gamma(G)$. Conversely, let $x \in X \backslash \alpha gcl_\gamma(G)$ which implies that $x \notin \alpha gcl_\gamma(G)$. Then, $\exists$ an αg-open set V of X containing $x \ni \gamma(V) \cap G = \phi$ implies that $\gamma(V) \subseteq X \backslash G$. Then, $x \in \alpha gint_\gamma(X \backslash G)$. Therefore, $X \backslash \alpha gcl_\gamma(G) \subseteq \alpha gint_\gamma(X \backslash G)$. Thus, $\alpha gint_\gamma(X \backslash G) = X \backslash \alpha gcl_\gamma(G)$.
(ii) Let $x \in \alpha gcl_\gamma(X \backslash G)$, then $\gamma(U) \cap X \backslash G \neq \emptyset\ \forall\ \alpha g$-open set U containing x. Suppose if $x \notin X \backslash \alpha gint_\gamma(G)$, then $x \in \alpha gint_\gamma(G)$ which implies that $\exists$ an αg-open set U of X containing $x \ni \gamma(U) \subseteq G$. Then $\gamma(U) \cap X \backslash G = \phi$ implies that $x \notin \alpha gcl_\gamma(X \backslash G)$, which is a contradiction. Therefore, $x \in X \backslash \alpha gint_\gamma(G)$. Hence $\alpha gcl_\gamma(X \backslash G) \subseteq X \backslash \alpha gint_\gamma(G)$. Conversely, let $x \in X \backslash \alpha gint_\gamma(G)$ which implies that $x \notin \alpha gint_\gamma(G)$. Suppose if $x \notin \alpha gcl_\gamma(X \backslash G)$, then $\exists$ an αg-open set V of X containing $x \ni \gamma(V) \cap (X \backslash G) = \phi$. Then $x \in \alpha gint_\gamma(G)$, which is a contradiction. Therefore, $x \in \alpha gcl_\gamma(X \backslash G)$. Hence $X \backslash \alpha gint_\gamma(G) \subseteq \alpha gcl_\gamma(X \backslash G)$. Therefore, $\alpha gcl_\gamma(X \backslash G) = X \backslash \alpha gint_\gamma(G)$.
(iii) & (iv) Follows from (i) and (ii).

Definition 3.10 Let A be a subset of a topological space (X, τ) and γ be an operation on $\tau_{\alpha g}$. Then αg_γ-interior of A is the union of all αg_γ-open sets contained in A and it is denoted by $\alpha g_\gamma int(A)$.

Proposition 3.11 Let A and B be subsets of X. Then

(1) $\alpha g_\gamma int(A) \subseteq A$

(2) $\alpha g_\gamma int(A)$ is an αg_γ-open set in X.

(3) $\alpha g_\gamma int(\phi) = \phi$ and $\alpha g_\gamma int(X) = X$.

(4) A is αg_γ-open iff $A = \alpha g_\gamma int(A)$.

(5) If $A \subseteq B$, then $\alpha g_\gamma int(A) \subseteq \alpha g_\gamma int(B)$

(6) $\alpha g_\gamma int(A) \cup \alpha g_\gamma int(B) \subseteq \alpha g_\gamma int(A \cup B)$

(7) $\alpha g_\gamma int(A \cap B) \subseteq \alpha g_\gamma int(A) \cap \alpha g_\gamma int(B)$

(8) If γ is αg-regular, then $\alpha g_\gamma int(A \cap B) \subseteq \alpha g_\gamma int(A) \cap \alpha g_\gamma int(B)$.

(9) $\alpha g_\gamma int\left(\alpha g_\gamma int(A)\right) = \alpha g_\gamma int(A)$

Proof: Obvious from the Definition 3.10.

Proposition 3.12 Let $A \subseteq X$. Then $\alpha g_\gamma int(A) \subseteq \alpha g int_\gamma(A)$.

Proof: Consider $x \in \alpha g_\gamma int(A)$. Then $x \in \cup \{O \subseteq X : O \subseteq A \; where \; O \in \tau_{\alpha g_\gamma}\}$. Then x belongs to atleast one αg_γ-open set, say O_1, contained in A. Since $x \in O_1$, $\exists$ an αg-open set V of X containing x ϶ $\gamma(V) \subseteq O_1 \subseteq A$. This implies that $x \in \alpha g int_\gamma(A)$. Hence $\alpha g_\gamma int(A) \subseteq \alpha g int_\gamma(A)$.

Remark 3.13 For any subset A of X, $\alpha g int_\gamma(A) \nsubseteq \alpha g_\gamma int(A)$.

Example 3.14 Let $X = \{a, b, c\}$ and $\tau = \{\emptyset, \{a, b\}, X\}$. Then $\tau_{\alpha g} = \{\emptyset, \{a\}, \{b\}, \{a, b\}, X\}$. Let $\gamma : \tau_{\alpha g} \to P(X)$ be an operation on $\tau_{\alpha g}$ defined by

$$\gamma(A) = \begin{cases} \{b, c\} & if \; A = \{b\} \\ A & otherwise \end{cases} \quad \forall \; A \in \tau_{\alpha g}$$

Then $\tau_{\alpha g_\gamma} = \{\emptyset, \{a\}, \{a, b\}, X\}$. Here for $A = \{b, c\}$, $\alpha g int_\gamma(A) = \{b\}$ whereas $\alpha g_\gamma int(A) = \emptyset$. Therefore, $\alpha g int_\gamma(A) \nsubseteq \alpha g_\gamma int(A)$.

Proposition 3.15 If $\gamma : \tau_{\alpha g} \to P(X)$ is an αg-open operation on $\tau_{\alpha g}$ and $A \subseteq X$. Then

a) $\alpha g int_\gamma(A) = \alpha g_\gamma int(A)$ and $\alpha g int_\gamma(\alpha g int_\gamma(A)) = \alpha g int_\gamma(A)$.

b) $\alpha g int_\gamma(A)$ is αg_γ-open in X.

Proof: a) Let γ be an αg-open operation on $\tau_{\alpha g}$. Let $x \in \alpha g int_\gamma(A)$. Then $\exists$ an αg-open set V of X containing x ϶ $\gamma(V) \subseteq A$. Since γ is an αg-open operation, $\forall$ αg-open set V containing $x \in X$, $\exists$ an αg_γ-open set U containing x ϶ $U \subseteq \gamma(V)$. i.e., $x \in U \subseteq \gamma(V) \subseteq A$. Then U is an αg_γ-open set which is contained in A and $x \in \cup \{U \subseteq X : U \subseteq A \; where \; U \in \tau_{\alpha g_\gamma}\}$. Therefore, $x \in \alpha g_\gamma int(A)$. Thus, $\alpha g int_\gamma(A) \subseteq \alpha g_\gamma int(A)$. Hence, by Proposition 3.12, $\alpha g int_\gamma(A) = \alpha g_\gamma int(A)$. Now, $\alpha g int_\gamma(\alpha g int_\gamma(A)) = \alpha g_\gamma int\left(\alpha g_\gamma int(A)\right) = \alpha g_\gamma int(A) = \alpha g int_\gamma(A)$.

(b) Follows from part (a) and Theorem 3.11.

Theorem 3.16 For any subset G of X,

(i) $\alpha g_\gamma Cl(X \backslash G) = X \backslash \alpha g_\gamma int(G)$

(ii) $\alpha g_\gamma int(X \backslash G) = X \backslash \alpha g_\gamma Cl(G)$

(iii) $\alpha g_\gamma int(G) = X \backslash \alpha g_\gamma Cl(X \backslash G)$

(iv) $\alpha g_\gamma Cl(G) = X \backslash \alpha g_\gamma int(X \backslash G)$

Proof: Obvious

Theorem 3.17 If $\gamma : \tau_{\alpha g} \to P(X)$ is an αg-regular operation on $\tau_{\alpha g}$. Then for any set $A \subseteq X$, the following holds:

a) $\alpha g_\gamma Cl(G) \cap U \subseteq \alpha g_\gamma Cl(G \cap U)$ $\forall$ αg_γ-open set U.

b) $\alpha g_\gamma int(G \cup F) \subseteq \alpha g_\gamma int(G) \cup F$ $\forall$ αg_γ-closed set F.

Proof: a) Let γ be an αg-regular operation on $\tau_{\alpha g}$. Let $x \in \alpha g_\gamma Cl(G) \cap U$ $\forall$ αg_γ-open set U. Then $x \in \alpha g_\gamma Cl(G)$ and $x \in U$. Consider an αg_γ-open set, say V, of X containing x. $\because \gamma$ is αg-regular, $U \cap V$ is αg_γ-open in X. By Theorem 3.38[7], $G \cap (U \cap V) \neq \phi$. This implies that $(G \cap U) \cap V \neq \phi$. Then $x \in \alpha g_\gamma Cl(G \cap U)$. Hence $\alpha g_\gamma Cl(G) \cap U \subseteq \alpha g_\gamma Cl(G \cap U)$ $\forall$ αg_γ-open set U.

b) From (a), $\alpha g_\gamma Cl(G) \cap U \subseteq \alpha g_\gamma Cl(G \cap U)$ $\forall$ αg_γ-open set U. Then $X \backslash (\alpha g_\gamma Cl(G \cap U)) \subseteq (X \backslash \alpha g_\gamma Cl(G)) \cup (X \backslash U)$. "By

Theorem 3.16, $X \backslash \alpha g_\gamma Cl(G \cap U) = \alpha g_\gamma int(X \backslash (G \cap U)) = \alpha g_\gamma int((X \backslash G) \cup (X \backslash U)) \subseteq \left(X \backslash \alpha g_\gamma Cl(G)\right) \cup (X \backslash U) = (\alpha g_\gamma int(X \backslash G)) \cup (X \backslash U)$. Hence, $\alpha g_\gamma int((X \backslash G) \cup (X \backslash U)) \subseteq (\alpha g_\gamma int(X \backslash G)) \cup (X \backslash U)$.

Remark 3.18 Theorem 3.17 fails when the operation γ is not αg-regular on $\tau_{\alpha g}$.

Example 3.19 Let $X = \{a, b, c\}$ and $\tau = \{\emptyset, \{a\}, \{b, c\}, X\}$. Let $\gamma : \tau_{\alpha g} \to P(X)$ be an operation on $\tau_{\alpha g}$ defined by

$$\gamma(A) = \begin{cases} A \cup \{c\} & if\ a \notin A \\ A & if\ a \in A \end{cases} \qquad \forall\ A \in \tau_{\alpha g}$$

Then $\tau_{\alpha g_\gamma} = \{\emptyset, \{a\}, \{c\}, \{a, b\}, \{b, c\}, \{a, c\}, X\}$ and γ is not αg-regular on $\tau_{\alpha g}$. If $G = \{a, c\}$ and $U = \{b\}$, then $\alpha g_\gamma Cl(G) \cap U = \{b\}$ whereas $\alpha g_\gamma Cl(G \cap U) = \emptyset$, so $\alpha g_\gamma Cl(G) \cap U \nsubseteq \alpha g_\gamma Cl(G \cap U)$.

Definition 3.20 Let A be a subset of a topological space (X, τ) and γ be an operation on $\tau_{\alpha g}$. Then αg_γ-kernel of A is defined as the intersection of all αg_γ-open sets containing A. It is denoted by $\alpha g_\gamma\text{-}ker\ (A)$.

$\alpha g_\gamma ker(A) = \cap \{U : A \subseteq U\ and\ U \in \tau_{\alpha g_\gamma}\}$.

Proposition 3.21 For any points x and y in X, $y \in \alpha g_\gamma ker(\{x\})$ iff $x \in \alpha g_\gamma Cl(\{y\})$.

Proof: If $y \in \alpha g_\gamma ker(\{x\})$, then $y \in \cap \{U : \{x\} \subseteq U\ and\ U \in \tau_{\alpha g_\gamma}\}$. i.e., y belongs to every αg_γ-open set containing $\{x\}$. This implies that $U \cap \{y\} \neq \phi$ $\forall$ αg_γ-open set U containing x. Then by Theorem 3.38[7], $x \in \alpha g_\gamma Cl(\{y\})$. Now, let $x \in \alpha g_\gamma Cl(\{y\})$. Then by Theorem 3.38[7], $U \cap \{y\} \neq \phi$ $\forall$ αg_γ-open set U containing x. This implies that y belongs to every αg_γ-open set containing $\{x\}$. Then, $y \in \alpha g_\gamma ker(\{x\})$.

Theorem 3.22 Let A be a subset of X. Then $x \in \alpha g_\gamma ker(A)$ iff $\alpha g_\gamma Cl(\{x\}) \cap A \neq \phi$ $\forall$ $x \in X$.

Proof: Let $x \in \alpha g_\gamma ker(A)$. Suppose if $\alpha g_\gamma Cl(\{x\}) \cap A = \phi$. Then, $x \notin X \backslash \alpha g_\gamma Cl(\{x\})$ which is an αg_γ-open set that contains A. This contradicts the fact that $x \in \alpha g_\gamma ker(A)$. Hence $\alpha g_\gamma Cl(\{x\}) \cap A \neq \phi$. Conversely, for $x \in X$, $\alpha g_\gamma Cl(\{x\}) \cap A \neq \phi$ and suppose $x \notin \alpha g_\gamma ker(A)$. Then, $\exists$ an αg_γ-open set V ϶ $A \subseteq V$ and $x \notin V$. Let $z \in \alpha g_\gamma Cl(\{x\}) \cap A \subseteq \alpha g_\gamma Cl(\{x\}) \cap V$. Hence V is an αg_γ-open set ϶ $z \in V$ and $z \in \alpha g_\gamma Cl(\{x\})$, implies that V intersects $\{x\}$.i.e., $x \in V$ which is a contradiction. Hence $x \in \alpha g_\gamma ker(A)$.

Proposition 3.23 Let A and B be subsets of X. Then

(a) $A \subseteq \alpha g_\gamma ker(A)$.

(b) If $A \subseteq B$ then $\alpha g_\gamma ker(A) \subseteq \alpha g_\gamma ker(B)$.

(c) $\alpha g_\gamma ker(\phi) = \phi$ and $\alpha g_\gamma ker(X) = X$.

(d) A is αg_γ-open then $A = \alpha g_\gamma ker(A)$.

(e) $\alpha g_\gamma ker(\alpha g_\gamma ker(A)) = \alpha g_\gamma ker\ (A)$.

Proof: (a) (b) & (c) Obvious.
(d) Let A be αg_γ-open. Let $x \in \alpha g_\gamma ker(A)$. Then x belongs to every αg_γ-open set containing A, which implies $x \in A$ for A is αg_γ-open. Thus $\alpha g_\gamma ker(A) \subseteq A$ and by (a), $A = \alpha g_\gamma ker(A)$.
(e) Suppose if $x \notin \alpha g_\gamma ker(A) = \cap \{U: A \subseteq U \text{ and } U \in \tau_{\alpha g_\gamma}\}$, then $\exists$ an αg_γ-open set U containing A ∋ $x \notin U$." Then $\alpha g_\gamma ker(U) = U$ for U is αg_γ-open. Hence we have $\alpha g_\gamma ker(A) \subseteq \alpha g_\gamma ker(U) = U$. By (b), $\alpha g_\gamma ker(\alpha g_\gamma ker(A)) \subseteq U$ which implies that $x \notin \alpha g_\gamma ker(\alpha g_\gamma ker(A))$. Therefore $\alpha g_\gamma ker(\alpha g_\gamma ker(A)) \subseteq \alpha g_\gamma ker(A)$. From (a) and (b), we get $\alpha g_\gamma ker(A) \subseteq \alpha g_\gamma ker(\alpha g_\gamma ker(A))$. Hence $\alpha g_\gamma ker(\alpha g_\gamma ker(A)) = \alpha g_\gamma ker(A)$.

Remark 3.24 The below example exemplifies that the obverse of Proposition 3.23 (d) is not true if γ is not αg-regular.

Example 3.25 Let X = {1,2,3}, τ ={∅, {1}, X} and $\tau_{\alpha g}$ ={∅, {1}, {2}, {3}, {1,2}, {1,3}, X}. Let $\gamma: \tau_{\alpha g} \to P(X)$ be an operation on $\tau_{\alpha g}$ defined by

$$\gamma(A) = \begin{cases} cl(A) & if\ 1 \notin A \\ A & if\ 1 \in A \end{cases} \qquad \forall\ A \in \tau_{\alpha g}$$

Then $\tau_{\alpha g_\gamma}$ = {∅, {1}, {1, 2}, {2,3}, {1,3}, X} and γ is not αg-regular on $\tau_{\alpha g}$. For A = {2}, $\alpha g_\gamma ker(A) = A$ but A is not αg_γ-open in X.

Proposition 3.26 Let γ be αg-regular. Then any set $A \subseteq X$ is αg_γ-open iff $A = \alpha g_\gamma ker(A)$.

Theorem 3.27 For any points x and y in X, the following are equivalent:

1. $\alpha g_\gamma ker(\{x\}) \neq \alpha g_\gamma ker(\{y\})$
2. $\alpha g_\gamma Cl(\{x\}) \neq \alpha g_\gamma Cl(\{y\})$

Proof: (1) ⇒ (2). Let $\alpha g_\gamma ker(\{x\}) \neq \alpha g_\gamma ker(\{y\})$. Then ∃ a point z in X ∋ $z \in \alpha g_\gamma ker(\{x\})$ and $z \notin \alpha g_\gamma ker(\{y\})$. Since $z \in \alpha g_\gamma ker(\{x\})$, by Proposition 3.21, $x \in \alpha g_\gamma Cl(\{z\})$. $z \notin \alpha g_\gamma ker(\{y\})$ implies that $\alpha g_\gamma Cl(\{z\}) \cap \{y\} = \phi$. i.e., $y \notin \alpha g_\gamma Cl(\{z\})$. Since $x \in \alpha g_\gamma Cl(\{z\})$, $\alpha g_\gamma Cl(\{x\}) \subseteq \alpha g_\gamma Cl(\{z\})$ implies that $\alpha g_\gamma Cl(\{x\}) \cap \{y\} = \phi$. Since, $\alpha g_\gamma Cl(\{x\}) \cap \{y\} = \phi$, $\alpha g_\gamma Cl(\{x\}) \neq \alpha g_\gamma Cl(\{y\})$.
(2) ⇒ (1). Let $\alpha g_\gamma Cl(\{x\}) \neq \alpha g_\gamma Cl(\{y\})$. Then ∃ a point z in X ∋ $z \in \alpha g_\gamma Cl(\{y\})$ and $z \notin \alpha g_\gamma Cl(\{x\})$. Since $z \notin \alpha g_\gamma Cl(\{x\})$, by Theorem 3.38[7], ∃ an αg_γ-open set U containing z ∋ $U \cap \{x\} = \phi$. This implies that U is an αg_γ-open set which contains z but not x. Similarly, Since $z \in \alpha g_\gamma Cl(\{y\})$, $U \cap \{y\} \neq \phi$ which implies U is an αg_γ-open set which contains y but not x, i.e., $x \notin \alpha g_\gamma ker(\{y\})$. Hence $\alpha g_\gamma ker(\{x\}) \neq \alpha g_\gamma ker(\{y\})$.

αg_γ-generalized closed sets

Definition 4.1 In a topological space (X, τ), $A \subseteq X$ is an αg_γ-generalized closed (concisely αg_γ-g.closed) set if $\alpha g cl_\gamma(A) \subseteq U$ whenever $A \subseteq U$ and U is αg_γ-open in (X, τ).

Proposition 4.2 Each αg_γ-closed set is αg_γ-g.closed but conversely the result does not hold.

Proof: Consider an αg_γ-closed set A and an αg_γ-open set U ∋ $A \subseteq U$. As A is αg_γ-closed, $A = \alpha g cl_\gamma(A)$ which implies that $\alpha g cl_\gamma(A) = A \subseteq U$. Thus A is αg_γ-g.closed.

Example 4.3 Let X = {1,2,3} and τ = {∅, {1}, {2}, {1,2}, {1,3}, X}. Then $\tau_{\alpha g} = \tau$. Let $\gamma: \tau_{\alpha g} \to P(X)$ be an operation on $\tau_{\alpha g}$ defined by $\gamma()$ = () ∀ ∈ . Then -closed sets = {∅, {2}, {1,3}, } and -.closed sets = (). The subset {1} is -.closed but not -closed.

Theorem 4.4 The following statements are equivalent for any subset in
(a) is -.closed in (,).
(b) ({}) ∩ ≠ ∀ ∈ ().
(c) () ⊆ ().

Proof : (a) ⇒ (b) Consider to be an -.closed set. Suppose that ∃ ∈ () ∋ ({}) ∩ = . This implies that ⊆ \({}). Since ({}) is -closed, \({}) is -open set that contains . ∵ is -.closed, () ⊆ \({}), which implies that ∉ (). This is a contradiction to ∈ (). Therefore ({}) ∩ ≠ ∀ $x \in \alpha gcl_\gamma(A)$.
(b) ⇒ (c) Let $x \in \alpha gcl_\gamma(A)$. Then $\alpha g_\gamma cl(\{x\}) \cap A \neq \phi$, ∃ a point z in $\alpha g_\gamma cl(\{x\}) \cap A$. Consider U as any αg_γ-open set ∋ $A \subseteq U$ then $z \in U$ and $z \in \alpha g_\gamma cl(\{x\})$. Then $U \cap \{x\} \neq \phi$ which implies $x \in U$ and hence $x \in \alpha g_\gamma ker(A)$. Therefore $\alpha gcl_\gamma(A) \subseteq \alpha g_\gamma ker(A)$.
(c) ⇒ (a) Consider an αg_γ-open set U ∋ $A \subseteq U$. Let $x \in \alpha gcl_\gamma(A) \subseteq \alpha g_\gamma\, ker(A)$. Since $x \in \alpha g_\gamma$ ker(A), $x \in U$. Therefore $\alpha gcl_\gamma(A) \subseteq U$. Implies A is αg_γ-g.closed in (X, τ).

Theorem 4.5 If a set $A \subseteq X$ is αg_γ-g.closed as well as αg_γ-open, then A is αg_γ-closed also.

Proof: Consider A to be αg_γ-g.closed. Then $\alpha gcl_\gamma(A) \subseteq A$. By Theorem 3.42[7], $A \subseteq \alpha gcl_\gamma(A)$. Hence $A = \alpha gcl_\gamma(A)$ which implies A is αg_γ-closed.
Theorem 4.6 If a set $A \subseteq X$ is αg_γ-g.closed then $\alpha gcl_\gamma(A) \backslash A$ does not contain any non-empty αg_γ-closed set.

Proof: Consider A to be an αg_γ-g.closed set. Suppose if ∃ a non-empty αg_γ-closed set F ∋ $F \subseteq \alpha gcl_\gamma(A) \backslash A$. Then $A \subseteq X \backslash F$ and $X \backslash F$ is αg_γ-open. Then $\alpha gcl_\gamma(A) \subseteq X \backslash F$ for A is αg_γ-g.closed. ⇒ $F \subseteq (X \backslash \alpha gcl_\gamma(A))$. Therefore $F \subseteq (\alpha gcl_\gamma(A)) \cap (X \backslash \alpha gcl_\gamma(A))$ which implies $F = \phi$. Hence no non-empty αg_γ-closed set is contained in $\alpha gcl_\gamma(A) \backslash A$.

Remark 4.7 The below example exemplifies that the obverse of Theorem 4.6 is not true always.

Example 4.8 Let $X = \{a, b, c\}$ and $\tau = \{\emptyset, \{a\}, \{b\}, \{a, b\}, \{a, c\}, X\} = \tau_{\alpha g}$. Let $\gamma: \tau_{\alpha g} \to P(X)$ be an operation on $\tau_{\alpha g}$ defined by

$$\gamma(A) = \begin{cases} A & if\ A = \{a, c\} \\ A & otherwise \end{cases} \qquad \forall\ A \in \tau_{\alpha g}$$

Then $\tau_{\alpha g_\gamma} = \{\emptyset, \{b\}, X\}$ and αg_γ-g.closed sets = $P(X) \backslash \{b\}$. Here, for $A = \{b\}$, $\alpha gcl_\gamma(A) = \{b, c\}$ and $\alpha gcl_\gamma(A) \backslash A = \{c\}$. Even though $\alpha gcl_\gamma(A) \backslash A$ does not contain any non-empty αg_γ-closed set, $\{b\}$ is not αg_γ-g.closed in X.

Remark 4.9 The obverse of Theorem 4.6 is true if γ is an αg-open operation.

Proof: Consider an αg_γ-open set U ∋ $A \subseteq U$. ∵ γ is an αg-open operation, $\alpha gcl_\gamma(A)$ is αg_γ-closed in (X, τ). Then $\alpha gcl_\gamma(A) \cap (X \backslash U) = F$(say) is αg_γ-closed in (X, τ). Since $X \backslash U \subseteq X \backslash A$, $F = \alpha gcl_\gamma(A) \cap (X \backslash U) \subseteq \alpha gcl_\gamma(A) \cap (X \backslash A), F \subseteq \alpha gcl_\gamma(A) \backslash A$. By Theorem 4.6, $F = \phi$ which implies that $\alpha gcl_\gamma(A) \cap (X \backslash U) = \phi$. Hence $\alpha gcl_\gamma(A) \subseteq U$. Therefore A is αg_γ. g-closed.

Corollary 4.10 Any αg_γ-g.closed set A of X is αg_γ-closed iff $\alpha gcl_\gamma(A) \backslash A$ is αg_γ-closed.

Proof: Consider A to be αg_γ-closed. Then $\alpha gcl_\gamma(A) = A$. This shows that $\alpha gcl_\gamma(A) \backslash A = \phi$. Therefore $\alpha gcl_\gamma(A) \backslash A$ is αg_γ-closed. Conversely, let A be αg_γ-g.closed and $\alpha gcl_\gamma(A) \backslash A$ be αg_γ-closed. Then by Theorem 4.6, $\alpha gcl_\gamma(A) \backslash A = \phi$. i.e., $\alpha gcl_\gamma(A) = A$ which implies A is αg_γ-closed.

Theorem 4.11 For each $x \in X$ either $\{x\}$ is αg_γ-closed or $X \backslash \{x\}$ is αg_γ-g.closed in (X, τ).

Proof: Suppose if $\{x\}$ is not αg_γ-closed, then $X\backslash\{x\}$ is not αg_γ-open. Then X is the only αg_γ-open set containing $X\backslash\{x\}$. Hence $\alpha gcl_\gamma(X\backslash\{x\}) \subseteq X$. Therefore $X\backslash\{x\}$ is αg_γ-g.closed in (X,τ).

Theorem 4.12 $\tau_{\alpha g_\gamma} = \tau^c_{\alpha g_\gamma}$ (αg_γ-open sets = αg_γ- closed sets) iff every subsets of X is αg_γ-g.closed in (X,τ).

Proof: Assume that $\tau_{\alpha g_\gamma} = \tau^c_{\alpha g_\gamma}$. Let U be an αg_γ-open set and A be any subset of X ∋ $A \subseteq U$. Now by Theorem 3.47 (ii) & (iv) [7], $\alpha gcl_\gamma(A) \subseteq \alpha gcl_\gamma(U) = U$ which implies A is αg_γ-g.closed in (X,τ). Conversely, assume that every subsets of X is αg_γ-g.closed in (X,τ). Consider an αg_γ-open set, U(say). ∵ U is αg_γ-g.closed and $U \subseteq U$, $\alpha gcl_\gamma(U) \subseteq U$. By Theorem 3.47 (i) [7], $U \subseteq \alpha gcl_\gamma(U)$. Therefore $U = \alpha gcl_\gamma(U)$. Hence U is an αg_γ- closed set.

REFERENCES

1. Asaad, B. A., and Ahmad, N., "Operation on semi generalized open sets with its separation axioms", AIP Conference Proceedings 1905, 020001 (2017).
2. Jankovic, D. S., "On functions with α-closed graphs", Glasnik Matematicki, Volume 18, Issue 3, 141-148 (1983).
3. Kasahara, S., "Operation compact spaces", Math. Japonica, Volume 24, Issue 1, 97-105 (1979).
4. Levine, N., "Generalized closed sets in topology", Rend, Circ, Math. Palermo, Volume 19, 89-96 (1970).
5. Maki, H., Devi, R., and Balachandran, K., "Associated topologies of generalized α-closed sets and α-generalized closed sets", Memoirs of the Faculty of Science Kochi University. Series A. Mathematics, Volume 15, 51–63 (1994).
6. Mershia Rabuni, J. J., and Balamani, N., "Operation approach on αg-open sets in topological spaces", (Submitted).
7. Njastad, O., "On some classes of nearly open sets", Pacific Journal of Mathematics, Volume 15, 961-970 (1965).
8. Ogata, H., "Operation on topological spaces and associated topology", Math. Japonica, Volume 36, Issue 1, 175-184 (1991).
9. Rehman, F. U. and Ahmad, B., "Operations On Topological Spaces-I", Mathematics Today, Volume 10, 30-36 (1992).
10. Roy, B. and Mukherjee, M. N., "A unified theory for R_0, R_1 and certain other separation properties and their variant forms", Boletim da Sociedade Paranaense de Matemática, Volume 28, Issue 2, 15-24 (2010).

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RESEARCH ARTICLE

Physiochemical and Biochemical Analysis of Vaippu Perungayam – A Research Article

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ABSTRACT

Siddha system of medicine is a distinct therapeutic science with many single, polyherbal and herbal and mineral formulations. Sarakuvaipu is a unique technique developed by our ancient siddhars which wasonly seen in siddha system of medicine. Naturally occurring *mooligai*(herbs), *karasaram* (salt), *uparasam*(minerals), *padanam* (arsenic compounds) and resins are made artificially called as Sarakku Vaipu which was said in many literature, by referring *Thiruvalluva Naayanaar Navarathina Vaithayam-800*. VaipuPerungayam was done using various herbal ingredients like fruits, leaves, tubers and volatile oil by following proper proposition and procedure. In day to day life perungayam (*Ferulaasa foetida*) is a commonly used as a food ingredient. Nowadays adulteration in Asafoetida is increasing tremendously due to increase in need. In order to avoid the usage of that, an alternate source for perungayam is needed. So the present study focused on the preparation of Vaippuperungayam which is used in food as a spice. In addition to that Vaipuperungayam is widely used in various ailments like Soothagasoolai (Dysmenorrhea), Gunmam (Peptic ulcer), Mandham (Indigestion), Paeruvayiru (Ascites), Valinoi (Vadham disorders). perungayam (Ferulaasafoetida) was also added as ingredient for many siddha medicines like Aagathiyar kuzhambu, Astathichooranam, Thalisathichooranam, Moosamparapatru, perungayachooranam. By testing the physiochemical and biochemical properties it shows the presence of carbonate, chloride, sulfate, sulphide, phosphate and zinc.

Keywords: Siddha, SarakuVaipu, Vaipu Paerungayam, Physiochemical, Biochemical Properties

INTRODUCTION

Siddha system is one of the very oldest systems of medicine found by various *siddhars* in southern part of India. Siddha system is based on three vital humors such as *vatham, pitham, kapham*. It consists of 32 internal and 32 external types of medicine. In addition to this, siddha system includes alchemy, vaithiyam, yogam, gnanam. Perungayam is the gum resin obtained from the rhizome and root of *Ferulaasa foetida*. Incision are made at the upper part of tap root of more than five year old plants and resin collected by scrapping in march, april after one or two days after a few weeks when it gets hardened, the process is repeated over and over.[1]It shows action such as stimulant, carminative, antispasmodic, expectorant, laxative, anthelminthic, diuretic [2]. It is widely used all over the world as flavoring spice in various types of foods. Medically it has an ability to cure various ailments. VAIPPU PERUNGAYAM is the artificial preparation of the *Ferula asafoetida* which mimics the original one.

MATERIALS AND METHOD OF VAIPPU PERUNGAYAM

VAIPPU MURAI / SARAKU VAIPPU

Sarakuvaippu is the process in which preparation of various metals, minerals in an artificial method. Siddhars are the one who did the technique sarakuvaippu preparation by using various medicinal products which resembles the properties of the natural one. Sarakuvaippu is seen only in siddha system of medicine among all the other system of medicines. It is done because of some original products are rarely available and many natural products are adulterated, even it may be too expensive, some extinct natural materials can also be prepared by sarakuvaippu. Vaippumuraigal is seen in various literatures such as *Panda vaipu, Sattamunnisarakuvaippu, Agathiyar amutha kalaiganam*, *Macha munniperunool800, Macha munni Thirumanthiram 800,* and many ulogam, padanam, karasaram, ubarasam are made using vaippumurai.

MATERIALS AND METHODS

DRUG SELECTION

In this research paper purified and prepared "**VAIPU PERUNGAYAM**" was taken from the Siddha literature "*Thiruvalluva Nayanar Navarathna Vaithiyam 800"* published by S.P.Ramachandiran, Thamarainoolagam,Chennai – 26 in the year of 1998.Page no: 116-117 [3].

உருவறிந்துபெருங்காயவைப்பதனை

யுணர்வறிந்துமொழியோதலால்

உலகருக்குஞ்சிவயோகிகட்குத்தவ

முனிவருக்கும்ரிஷியனைவற்குங்

கருவறிந்துபனவெல்லம்ரெண்டுபலஞ்

சுக்குரெண்டுபலமாதலாற்

கஞ்சநாதமெனுங்குங்கிலீகமது

கருதிடில ஒத்த ருதன்பலமதுஞ்

சுரூபமாகுமிவைவெள்ளுள்ளியினிட

கத்தமாந்தயிலமிருபலம்

சூட்டும்வாலுளுவைவித்ததுமெனிலுந்

தூக்குவாயுளுந்தின்மாவதும்

பருவமாகுமாறுபலமுமாகுவிதம்

பாருங்கூவிளததின்பழமதும்

பாகமெட்டுபலமாவதுமுரலிற்

பதமதாகவுமேதாக்கிடே

தாக்கிடுங்கருங்கல்லுரலிலிட்டுயிடித்

தானதுமெழுகுபோல்வருந்

தனதுரூபமதிற்கடுகதின்றயிலந்

தாக்கிடுமதையேஜாடிப்பின்

ஆக்கிடும்பதமில்மேழரோமமது

வதுருபோலலனைத்தூளதாய்

அதனிலும்விடுமைபோலுஞ்சாடி

விடுயிதைவழித்துவதின்தோடதிற்

பாக்கிடும்அளவுதான்கொடுத்துபுலி

பாதாளக்குழிநெல்லுமியினிற்

பதியமண்டலமும்பாவித்தேவிடுநீற்

பாரும்பாரும்பண்டதர்கள்தான

நீக்கிடுமதுநாட்சென்றெடுத்துவிடு

நீட்புவிக்குமதுவாடைபார்

நின்றுலாவியவிழ்தங்களானதுவில்

நிகழ்த்திடுவீர்வைத்தியர்களே.

COLLECTION OF THE RAW DRUGS

The above mentioned raw drugs were purchased from a well reputed siddha [country] shop. Vilvapazham were collected from nearby garden.

IDENTIFICATION AND AUTHENTICATION OF THE RAW DRUGS

All the raw drugs and fruits were identified and authenticated by botanists and Gunapadam experts in Sri Sairam Siddha Medical College and Research centre, Chennai-44.

PURIFICATION OF THE RAW DRUGS

- Panaivellam- cleaned the dust particles
- Sukku- outer skin were removed and dried.
- Vellaikunkiliyam - cleaned and made into powder
- Poonduthailam- was prepared from garlic

- Vaalluzhuvaivithu– seeds were purified.
- Vilvapazhasathai- seedswere removed and mesocarp dried in sunlight
- Ulunthumavu - cleaned and made into powder.
- Kadakuennai– original oil was used.

PREPARATION OF THE MEDICINE

All the raw drugs were purified by using the method above, drugs Such as 70 grams of Sukku *[Zingiber officinale]* were added to the Kalural (Ebony Mortar and Pestle) and made into fine powder mix it with 210 grams of Ulunthumavu *[Vigna mungo]* and vasthrakayam (filtered using cloth) were done.Add 35grams of Vellaikunkiliyam *[Vateria indica],* 70 grams of Panaivellam [palm jaggery], grind well and add 210 grams of Vaalluzhuvaivithu *[Celastrus paniculatus]* Which turns oily texture and consistency. Then add 280 grams of Vilva pasha sathai *[Aegle marmelos],* 70 ml of Poonduthailam (required amount of garlic *[Allium sativum]* were peeled off the papery skin and then crushed and placed into the cloth and firmly introduce into the flame,then the extract starts to ooze which was collected in a vessel), required amount of Kadakuennai [mustard oil] and grind well made into fine paste form.[3]

PUDAM MURAI

There are various pudam methods used in siddha system of medicine like Ummipudam, Thaniyapudam, Suriyapudam, Santhirapudam, Panipudam. The prepared Vaippu Perungayamis burried under nelummi [paddy husk] and it is taken after forty days.

PURIFICATION OF THE VAIPUPERUNGAYAM

The "Vaipu Perungayam" were purified by frying method (frying in oil) as per siddha classical literature. A mud pot was taken and it was poured with required quantity of oil or ghee and add Vaipu Perungayam into the oil wait until it fry and use it for food and medicinal preparations.

STORAGE OF THE DRUG

The prepared test drugs were stored in a clean, air tight glass container.

PERUNGAYAM CONTAINING MEDICINES

Perungayayam (*Ferulaasa foetida*) was also added as ingredient for many siddha medicines like Aagathiyar kuzhambu, Astathichooranam, Thalisathichooranam, Moosamparapatru,Perungayachooranam.

INDICATIONS

Soothagasoolai (Dysmenorrhea), Gunmam(Peptic ulcer), Mandham(Indigestion),Paeruvayiru (Ascites), Valinoi (Vadha disease).

CHEMICAL ANALYSIS

After forty days vaippuperungayam was sent to Noble Research Solutions and the physiochemical and biochemical analysis were done [4,5].

PHYSIOCHEMICAL EVALUATION

Percentage Loss on Drying

Test drug was correctly weighed in evaporating dish. Sample were made to dry at 105°C for 5 hours and it was weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated in the furnace at a temperature of 400 ºC until it turns white in color which indicates the carbon absence. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6minutes. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

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BIOCHEMICAL ANALYSIS

ANALYTICAL INVESTIGATION ON TEST FOR ACID RADICALS

Test for Specific Acid Radicals

Test for Carbonates

To 1 ml of the test solution about 1 ml of concentration (conc.) HCl was added and observed for the formation of brisk effervescence indicates the presence of Carbonates.

Test for Chlorides

To 2 ml of test solution, about 1 ml of Silver Nitrate solution added and observed for the white precipitate which shows chlorides presence..

Test for Sulfates

To 1 ml of the test sample add diluted H_2SO_4 till effervescence ceases followed by this about 1ml of Barium chloride solution were added and observed for the appearance of white precipitate indicates the presence of Sulfates.

Test for Sulfides

To 1 ml of the test sample about 2 ml of HCl was added with slight warming the mixture and observed the formation of colorless gas with the smell of rotten egg indicates the presence of Sulfides.

Test for Phosphates

To 2 ml of test solution treated with 2 ml of Ammonium Molybdate solution followed by addition of 2 ml of concentrated Nitric acid and observed the formation of yellow precipitate indicates the presence of Phosphates.

Test for Fluoride and Oxalate

To 2 ml of the test solution about 2 ml of dilute Acetic acid and 2ml of Calcium Chloride solution was added and observed theformation of white precipitate indicates the presence of Fluoride/ Oxalate.

Test for Borates

2ml of the test solution was added with Sulphuric acid and 95% alcohol followed by exposure to flame and observed theappearance of green flame indicates the presence of Borates.

Test for Nitrates

0.5 ml of test solution heated with Copper turning followed by addition of Sulphuric acid and observed the appearance of reddish brown gas indicates the presence of Nitrates.

ANALYTICAL INVESTIGATION ON TEST FOR BASIC RADICALS

Test for Specific Basic Radicals

Test for Lead

1 ml of the test solution added with 2 ml of Potassium Chromate solution and observed the formation of yellow precipitate indicates the presence of Lead.

Test for Arsenic

1 ml of the test solution added with 2 ml of 10% (2N) Sodium hydroxide (NaOH) solution and observed the formation of brownish red precipitate indicates the presence of Arsenic.

Test for Mercury

1 ml of the test solution added with 2 ml of 10% (2N) Sodium hydroxide solution and observed theformation of yellow precipitate indicates the presence of Mercury.

Test for Copper

1 ml of the test solution added with 1 ml of Ammonium hydroxide (NH_4OH) solutionand observed theformation of blue precipitate indicates the copper presence.

Test for Ferric

To 1 ml of test solution, about 2 ml of Potassium Ferrocyanide was addedand observed theformation of blue precipitate indicates the presence of Ferric.

Test for Ferrous

To 1 ml of test solution, about 1 ml of Potassium Ferric Cyanide solution was addedand observed theformation of blue precipitate indicates the presence of ferrous.

Test for Zinc

1 ml of the test solution added with 2 ml of Sodium hydroxide (NaOH) drop wise until indication appearsand observed theformation of white precipitate indicates the presence of Zinc.

Test for Silver

1 ml of the test solution was added with 1 ml of conc. HCl followed by appearance of curdy white precipitate. The precipitate boiled in water and it doesn't dissolve in water. Add NH_4OH solution in it and add 1 ml of dilute $HNO_3$and observed theformation of curdy white precipitate indicates the presence of Silver.

Test for Magnesium

1 ml of the test solution added with 2 ml of Sodium hydroxide (NaOH) drop wise until indication appearsand observed theformation of white precipitate indicates thepresence of Magnesium.[4,5]

RESULT AND DISSCUSSION

Result of physiochemical analysis of vaippu perungayam (Table 4), Result of biochemical analysis of vaippu perungayam (Table 5), Result for specific acid radicals (Table 6).

DISCUSSION

Since unadulterated Asafoetida has an extreme flavor, it is blended in with starch and gums. It is sold as compound Asafoetida (brick form) powder form or tablet form. It contains Asafoetida resin (30%) just as rice flour (50%) and gum arabic (to forestall lumping). It is utilized to give flavor in food , anti-infection in medicine, anti-flatulent,the respectability which are added contains grit, sand, dirt, filth, chalk, soapstone and unfamiliar pitch. These respectable qualities caused the stomach looseness of the bowels, poisioning,renal, regenerative, musculoskeletal, neurological, ocular and etc. Hencevaippu perungayam is can be used as the alternative for adulterated asafetida [6].

CONCLUSION

The present study shows the preliminary screening of Physio-chemical, Biochemical, Acid and Basic radicals screening analysis shows the presence of:

- Carbonates
- Sulfates
- Sulphides
- Phosphates
- Arsenic
- Zinc

Will provide footprints for further clinical studies.

REFERNCES

1. The siddha pharmacopoeia of India, part – 1, first edition, Government of India, Ministry of Health and Family Welfare, Department of AYUSH.
2. KS. Murugesamuthaliyar, Siddha Materia Medica part – 2, department of Indian medicine and homeopathy, Chennai -106.
3. S.P Ramachandran, Thiruvalluva Nayanar Navarathinavaithiya sindhamani – 800, December 1998, Thamarainoolagam, Chennai – 26.
4. India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, 2014.
5. Pharmacopoeia Laboratory for Indian Medicine (PLIM) Guideline for standardization and evaluation of Indian medicine which include drugs of Ayurveda, Unani and Siddha systems, Department AYUSH ,Ministry of Health & Family Welfare, Govt. of India.
6. A study on the contamination of communal used species of power [Asafoetida] In India, international journal of research in engineering and applied science, vol 6 issue 9 September – 2016.

Table : 1.Ingredients

NAME OF DRUGS	BOTANICAL NAME	QUANTITY[in grams]
Panaivellam	*Palmyra palm*	70
Sukku	*Zingiber officinale*	70
Vellaikunkiliyam	*Vateria indica*	35
Poonduthailam	*Allium sativam*	70
Vaalluzhuvaivithu	*Celastruspaniculatus*	210
Vilvapazhasathai	*Aegle marmelos*	280
Ulunthumavu	*Vigna mungo*	210
Kaduguennai	*Brassica nigra*	Required quantity

Table :2 Physiochemical evaluation

State	Semisolid
Nature	Moderately smooth
Odor	Characteristic
Touch / Consistency	Greasy
Flow Property	Non- free flowing
Appearance	Dark Brownish

Table :3 Solubility profile

S.No	The Solvent Used	Solubility or Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	DMSO	Soluble

Table:4 Physiochemical analysis

S.No	Parameter	Mean (n=3) SD
1.	*Loss on Drying at 105 °C (%)*	17.03 ± 0.49
2.	*Total Ash (%)*	11.93 ± 1.41
3.	*Acid insoluble Ash (%)*	0.12 ± 0.08
4.	*Alcohol Soluble Extractive (%)*	14.33 ± 3.51
5.	*Water soluble Extractive (%)*	33.67 ± 2.08

Table:5 Acid radicals

S.No	Test for Specific Acid Radical	Indication / Observation	Inference	Result
1.	**Test for Carbonates**	Formation of brisk effervescence	Presenceof brisk effervescence	**Positive**
2.	**Test for chlorides** .	Appearance of White precipitate	Absence of White precipitate	Absence
3.	**Test for sulfates**	Appearance of white precipitate	Presence e of white precipitate	**Positive**
4.	**Test for sulfides**	Formation of colorless gas with the smell of rotten egg	Presence of rotten egg smell	**Positive**
5.	**Test for phosphates**	Formation of yellow precipitate	Presence of yellow precipitate	**Positive**

6.	**Test for Fluoride and Oxalate**	Formation of white precipitate	Absence of white precipitate	Negative
7.	**Test for Borates**	Appearance of green flame	Absence of green flame	Negative
8.	**Test for Nitrates**	Appearance of reddish brown gas	Absence of reddish brown color	Negative

Table:6. Basic radicals

S.No	Test for Specific Basic Radical	Indication / Observation	Inference	Results
1.	**Test for Lead**	Formation of yellow precipitate indicates the presence of lead.	Absence of yellow precipitate	Negative
2.	**Test for Arsenic**	Formation of brownish red precipitate indicates the presence of Arsenic	Presence of brownish red precipitate	**Positive**
3.	**Test for Mercury**	Formation of yellow precipitate indicates the presence of mercury.	Absence of yellow precipitate	Negative
4.	**Test for Copper**	Formation of blue precipitate indicates the presence of copper.	Absence of blue precipitate	Negative
5.	**Test for Ferric**	Formation of blue precipitate indicates the presence of ferric.	Absence of blue precipitate	Negative
6.	**Test for Ferrous**	Formation of blue precipitate indicates the presence of ferrous	Absence of blue precipitate	Negative
7.	**Test for Zinc**	Formation of white precipitate indicates the presence of Zinc.	Presence of white precipitate	**Positive**
8.	**Test for Silver**	Formation of curdy white precipitate indicates the presence of silver.	Absence of curdy white precipitate	Negative
9.	**Test for Magnesium**	Formation of white precipitate indicates the presence of Magnesium.	Absence of white precipitate	Negative

Fig : 1 Sample

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REVIEW ARTICLE

Anti-Diabetic Property of Siddha Herbal Compound Drug *Panchathuvarpi*–A Review

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder, in which hyperglycaemic state is due to the deficiency in insulin secretion or inadequate response of this hormone. Type 1 (T1DM, autoimmune disorder) is characterised by an inability to produce insulin because of the partialor total destruction of β-pancreatic cells. The most common type 2 (T2DM), is an inadequate secretion or resistance to the insulin by the cells. Worldwide usage of medicinal plants for the management of T2DM is rising nowadays and the hypoglycaemic properties in the herbals are due to the presence of phyto-constituents like polyphenols, terpenoids, flavonoids, glycosides and saponins. Oxidative stress also more related with DM. In siddha, according to *thrithodam* theory, the derangement of *Pitham*and *Kapha* humour is the causative factorfor the development and complications of the diabetes. To neutralise the vitiated *Pitham* and *kapha*humour, plants which having astringent taste have been prescribed. The sensation of astringency depends on the tannins, a polyphenol compound which is found in bud, seeds, leaves, roots and stem tissues. In this view, the present study is aimed to review the anti-diabetic properties of Siddha herbal compound drug *Panchathuvarpi*(*Thokaisarkkukal*) which comprises five astringent herbs *Ficusbenghalensis* (*Aal*), *Ficusreligiosa* (*Arasu*), *Ficusracemosa* (*Atti*), *Ficusmicrocarpa* (*Itti*) and*Syzygiumcumini* (*Naavel*).They have been widely used in Indian traditional medicine for the management of DM due to its hypoglycaemic and antioxidant property. From this review of literature and scientific evidences including published articles stated that, the ingredients in *Panchathuvarpi* possess regulation of intestinal tract enzymatic activities, inducing insulin secretion, Enhancing insulin sensitivity, increasing hepatic glycogen synthesis, declining carbohydrate absorption, increasing peripheral glucose uptake and improving antioxidant

status. This study concluded that, the ingredients of *Panchathuvarpi* having good anti-diabetic property due to the presence of various phyto- constituents and it can be used for the management of DM.

Keywords: Diabetes mellitus, Siddha medicine, Astringent, *Panchathuvarpi,* Anti-diabetic property

INTRODUCTION

Diabetes is a metabolic disorder in which defects in the carbohydrate, protein and fat metabolism characterized by chronic hyperglycemia resulting from faults in insulin secretion, insulin function, or both. The chronic elevation in blood sugar is associated with long-term damage, dysfunction, and failure of different organs, specially the nerves, eyes, blood vessels, kidneys, heart. Diabetes mellitus was earlier grouped into two types but most of the researchers now classify them into four types**.**Type I (Insulin Dependent Diabetes Mellitus) is an autoimmune disorder where antibodies destroy beta cells of the islets of Langerhans leads to the absolute deficiency of insulin. Usually happened in young children and adolescents**.** Type II(Non-Insulin Dependent Diabetes Mellitus) is due to the reduced sensitivity of the tissues to insulin and impaired regulation of insulin secretion. Type III or Secondary diabetes is due to causes like pancreatectomy, drugs and non-pancreatic diseases.Type IV is a Gestational Diabetes which occurs around 20 -24 weeks of pregnancy due to the insulin resistance caused by the placental hormones [1]**.**Age,genetic factors, dietary habits, sedentary lifestyles, obesity are the factors responsible for the development of diabetes mellitus. Acute complication includes hyperglycaemia, hypoglycaemia and ketoacidosis. Retinopathy, nephropathy, neuropathy, coronary artery disease,peripheral arterial disease, and stroke are the chronic complication of diabetes mellitus due to the prolonged elevation of glucose in the blood. Still now the scientific communities are struggling to identify the biologically active compounds with no or minimal side effects for the management of diabetes mellitus. Even before the active components of herbals explored,herbal medicines are broadly prescribed to treat the diabetes.

Siddha system follows a unique methodology to diagnosis (Siddha pathology) and determine of etiology of the disease (*NoimudalNaadal*) and selecting proper treatment especially from medicinal herbals based on their taste.In siddha system of medicine certain drugs or parts of the herbals are compoundly classified based on their similar taste or potency or action or aromatic properties which is called as *Thokaisarakkukal*.Likewise*Panchatuvarpi* is a compound herbal drug which comprises barks of *Aal (Ficus benghalensis), Arasu (Ficus religiosa), Atti (Ficus racemosa), Itti (Ficus microcarpa)*and *Naaval (Syzygium cumini).* Various pharmacological studies on these herbs have been conducted in past decades. This review summarize the traditional knowledge about the *Panchathuvarpi*for the management of DM and collect the various scientific evidences to support the traditional usage of *Panchathuvarpi.*

Diabetes in Siddha

Siddh are categorise the diabetes disease under the classification of *Neerinaiperukkal Noikal* (Excessive urination). Synonyms found in siddha literature to mention the diabetes are *Madhumegam ,Innippuneer, Neerizivunoi*.*Thrithodam* theory is the main principle in siddha for diagnosis, treatment and assessing the prognosis. *Vatham*, *Pitham* and *Kapham* are the three bio energies responsible for balancing the physical and functional status of the human body.Any derangement in these three humours leads to pathological condition. According to Siddha Concept, the *Pitham*(Fire) which is responsible formetabolic functions and digestion which initiate the Diabetes by vitiated in its nature due to the changes in life style and unwanted dietary habits(*Unavaathi Seyal*) and further progression leads to derangement of *Kapha* humor (water) results in development of diabetes and its complications. Deranged *pitham* leads to excessive thirst and increased appetite. Diabetic complications such as retinopathy, nephropathy and neuropathy are due to the accumulated *kapham* in human body.To balancing the three humours in human body, the taste which consume plays a vital role.Each taste is composed of two elements. Astringent is the taste out of six tastes composed mainly of air and earth element has the potency to neutralize the deranged *Pitham* and *kapham*. With the

purpose of balancing the deranged *pitham* and *kapham*, the taste *thuvarpu* (astringent) are found to be acceptable for the choice of anti-diabetic herbs.

Panchathuvarpi

Panchathuvarpi is the name given to the barks of five astringent of *Aal (Ficus benghalensis), Arasu (Ficus religiosa), Atti(Ficus racemosa), Itti (Ficus microcarpa)* belongs to the family of *Moraceae, Naaval (Syzygium cumini)* from *Myrtaceae* family.Different parts of the plants especially bark, seeds, fruit, leaves, latex have the promising effect on treating the various ailments. A number of herbal formulations are also prepared in combination of with this plant showed potential anti-diabetic activity and are used regularly by diabeticpatients. Some of the preparations mentioned in the literatures are listed below (Table no; 1)

Mechanism of Anti-diabetic Drugs

The drug or agent has anti-diabetic action has the property of Increased insulin release from pancreas, Increased sensitivity to insulin, Decreased glucagon release, Delay gastric emptying, Decreased appetite, Decreased hepatic gluconeogenesis, Increased glucose transport into the tissues, Decreased glucose absorption, Increased Glucose excretion, Inhibit sodium glucose co-transporter -2 in kidneys, Decreased hydrolysis of disaccharides[1]**.**The anti - diabetic drug should be investigated for the following mechanism such as increased glucose uptake in skeletal muscle, inhibition of glucose absorption in the intestinal tract and regulation of the glucose homeostasis and antioxidant activity[8] for providing the effective treatment to the community. The mechanism is described in fig no; 1.

Phytochemicals of *Panchathuvarpi*

Several phytochemicals such as phenols, flavonoids, lignans are responsible to ease the diabetes complications. The insulin sensitivity is enhanced by the phytochemicals having antioxidant and free radical scavenging activity[9]. Here the phytochemicals found in the *Panchathuvarpi* are listed below (Table no:2)

Anti-diabetic property of *Panchathuvarpi*

There are lot of scientific studies has been conducted on the herbal comes under the *Panchathuvarpi*for its Anti diabetic property.

Aal (Ficus benghalensis)

- Kumar et al.,conducted a study ona Dimethoxyderivative of leucocyandin 3-O-beta-d-galactosyl cellobioside from the bark of *F.benghalensis* in normal and moderately diabetic rats at dosage of 250 mg/kg for a 2hr period of oral administration. The results obtained from the experiment is Insulin degradative processes Inhibition, Blood sugar lowering action and serum insulin elevation [17].
- Patel DK Et al., reviewed the plants having insulin mimic propery and stated that the Dimethoxy ether of leucopelargonidin-3- O-alpha-L rhamnoside from the *F.benghalensisbark* at a dose of 100 mg/kg, p.o. in alloxan induced diabetic dogs during a period of 2 hour, shows hypoglycaemic and insulin mimetic activity [18].
- Mahalingam Gayathri et al.,stated that ,the blood glucose Control,restored glycolytic enzymes and antioxidant activity of *Ficusbenghalensis* bark in streptozotocin induced diabetic rats at a dose of 500mg/kg/day[19].
- Shukla et al., conducted the research work on Hypoglycaemic effect of the water extract of *Ficusbenghalensis* in alloxan recovered, mildly diabetic and severely diabetic rabbits at a single dose of 50 mg/kg/day for three days. This study concluded the improvement in glucose tolerance in alloxan recovered rabbits and in mildly and severely diabetic rabbits ,there is a fall in fasting blood glucose and an improvement in glucose tolerance [20].
- The phytochemical Leucoperalgonid in from the *Ficusbenghalensis* exhibit best docking with aldose reductase receptor in molecular docking analysis of medicinal plants with type 2 diabetes mellitus targets, stated by Singh P et al [21].

- Singh et al., and Singh AB et al., reported the Anti-diabetic effect of aqueoes and ethanolic extract of *Ficus benghalensis* aerial roots in STZ-induced diabetic rats and mice respectively. It shows Expression of insulin geneand Decreases the blood glucose level at the dose levels 300 mg/ kg ,50 mg/kg p.o accordingly [22,23].

Arasu (Ficus religiosa)

- Panditet al., observed that at the dose of 50 mg/kg,the aqueous extract of *F. religiosa* bark exhibited maximum glucose lowering effect in STZ-induced diabetic rats and has the antioxidant activity by reducing the oxidative stress [24].
- Ambikaet al., isolated the phytosterolin from the bark of the *F. religiosa* and administered at the dose of 5, 7.5 mg/kg, i.v. and 25 mg/kg,p.o. in fasting rabbit. Phytosterolin treatment decreased the blood glucose level, after 2 h of 5 and 7.5 mg/kg; i.v. and after 4 h of 25 mg/kg; p.o. dose. The effect of phytosterolin at 7.5 mg/kg; i.v. was found to be equipotent to the standard drug to Ibutamide. The bioactive compound phytosterolinis found to be responsible for the hypoglycemic effect of the bark [25].
- Kirana H et al., reported the Anti-diabetic effect of the aqueous extract of *F. religiosa* bark on streptozotocin (STZ)-induced type 2 diabetes in rats treated with the doses of 100 and 200 mg/kg; p.o. Treatment results increased the body weight and decreased the fasting blood glucose level in type 2 diabetic rats. Moreover, the extract treatment reduces the oxidative stress by controlling the enzymatic activity of glutathione peroxidase , catalase, and superoxide dismutase [26].
- The water extract of root bark of *F. religiosa* are found to exhibit hypoglycaemic activity, on oral administration to rabbits at the dose level of 2.5 g,studied by Brahmachariet al [27].

Atti (Ficus racemosa)

- Keshariet al., isolated the flavonoids from the bark of *Ficus racemosa* in Streptozotocin-induced diabetic rats.At 100mg/kg dose level, exhibit lower blood glucose and antioxidant activity. Moreover the molecular docking studies also confirmed that isolated flavonoids have anti-diabetic potentials by binding to PPARγ and GLUT1 receptors[28].
- Joshi et al., study showspotent blood glucose lowering and antioxidative activity against STZ induced diabetes rats at a dose of 400 mg kg^{-1}*F. racemosa* bark extract and Sophia et al observed thepotent anti-diabetic activity by restoring the blood glucose to the normal range and hypolipidemic effects at the dose level 300 mg/kg bw in Alloxan-induced diabetic rats [29,30].
- Gul-e-Rana et al., conducted a clinical trial on Hypoglycemic effect of *Ficus racemosa* bark in a group of diabetic people taking oral hypoglycemic drug with the bark of *Ficus racemosa* (about 100 mg) two times for 15 days – Reduction in Fasting and post prandial blood glucose and no liver and renal toxicity observed.[31]
- The phytochemical Kaempferol from the *Ficus racemosa* exhibit best docking with aldose reductase receptor in molecular docking analysis of medicinal plants with type 2 diabetes mellitus targets,stated by Singh P ,et al.[21]
- Ravichandiran et al., carried out a study on Oral administration of tannin from *Ficus racemos* are verse the increased blood glucose, restored the insulin and high density lipoprotein in the serum in high fat diet and streptozotocin treated diabetic rats at the dose of 100 & 200 mg/kg. Bhaskara Rao evaluated the the hypoglycemic effectof methanol extract of the *Ficus racemosa* stem bark.At 200 and 400 mg/kg p.o.doses exhibit significant hypoglycaemic activity in alloxan diabetic rats [32,33].
- Faiyaz Ahmed et al., reported the inhibitory effect on carbohydrate hydrolyzing enzymes alpha-amylase, alpha-glucosidase, beta-glucosidase, and sucrase in a dose-dependent manner from the in-vitro studies on *F. racemosa* bark [34].
- Jahan et al., studied the effects of *Ficus racemosa* fruit extract at the dose of 1.25 g/ kg on normal, type 1 and type 2 diabetic model rats .The experiment shows the delayed absorption of glucose in the intestine [35].

Itti (Ficus microcarpa)

- The methanol and ethanol extracts of *F. microcarpa* leaves was evaluated for anti-diabetic property against alloxan-induced diabetic rats at the dosage level 100 & 200 mg/kg by Asok Kumar et al .The study result shows reducing blood glucose level and increased insulin level in experimental rats [36].

Naaval (Syzygium cumini)

- The study conducted by Saravanan G et al.,on Aqueous *S. cumini* bark extract in streptozotocin induced diabetic rats at the dose of 300 mg/kg indicate the elevated levels of plasma insulin and C-peptide and reduction in urine sugar [37].
- Schossler DRC et al.,found β-cell regeneration in 1g/kg extract of *S.cumini* bark treated experimental rats in which the β-cells are destroyed by alloxan [38].
- A. Kumar et al., stated that the compound 'Mycaminose'(50 mg/kg) and ethyl acetate and methanol extracted *S. cumini* seed (200 and 400 mg/kg) produced reduction in blood glucose level in streptozotocin (STZ)-induced diabetic rates [39].
- Ethanolic extract of *S. cumini* seed in streptozotocin induced diabetic rats at the dose of 200 mg/kg showed increase in insulin level and glycosylated hemoglobin level close to the normal level, reported by Chaturvedi A et al [40].
- Prince PSM et al., studied the Oral administration of *S. cumini* seed extract in alloxan induced diabetic rats at the dose of 100 mg kg^{-1} resulted in reduction in blood and urine sugar level [41].
- Prince PSM et al., experimental study state that the aqueous extract of *S. cumini* seed in alloxan induced diabetic rats exhibited hypoglycaemic action, decreased free radical formation at the dose level of 5.0 g/kg body weight [42].

CONCLUSION

Siddha herbal formulations have been established and used for the management of diabetes since ancient times. Especially the herbals belongs to the *Panchathuvarpi* has been prescribed to treat the diabetes and its complication based on their astringent taste to neutralize the deranged humours in *Madhumegam* (diabetes). Scientifically, all the herbals in *Panchathuvarpi* exposed anti-diabetic effect against alloxan and streptozotocin induced diabetic animals by inhibiting glucose absorption, improve the secretion of insulin, enhancing the glucose uptake and regenerating the pancreatic β-cells, improving antioxidant levels. This review also delivers sufficient knowledge to researchers to understand the anti-diabetic actions of *Panchathuvarpi* and mechanism of anti-diabetic action. Furthermore, it provides a traditional and scientific basis for developing the effective anti-diabetic drug from plant sources to reduce the burden of the synthetic drugs.

REFERENCES

1. PadmajaUdhayakumar,Medical pharmacology ,seventh edition, CBS Publishers & Distributors) pg no ;504
2. Murugesamuthaliyar,Gunapadam First part – Mooligai, Indian Medicine and Homeopathy,2013
3. SomasundaramS,Taxonomy of angiosperms(medicinal botany part -2),Elangovan publisher,6 th edition ,2015
4. UthamarayanK.S,SiddharAruvaiMaruthuvam, Indian Medicine and Homeopathy, 6th edition,2013.
5. Mohan R.C,PatharthaGunaSinthamani,Tamarai library,5 th edition 2018.
6. KuppusamymudhaliyarK.N,SiddhaMaruthuvamPothu, Indian Medicine and Homeopathy,8 th edition 2016,pg no;531
7. Hakeem Abdullah saibu, Mega nivaranapotiniennumNeerizhivumaruthuvam,Tamarai library publication,June 1998.
8. El-Abhar HS, Schaalan MF. Phytotherapy in diabetes: Review on potential mechanistic perspectives. World J Diabetes 2014; 5(2): 176-197

9. Bacanli M, Dilsiz SA, Başaran N, Başaran AA. Effects of phytochemicals against diabetes.Adv Food Nutr Res. 2019; 89:209-238.
10. Hafiz Abdul Khaliq, A review of pharmacognostic, physicochemical, phytochemical and pharmacological studies on *Ficusbenghalensis* L., Journal of Scientific and Innovative Research 2017; 6(4): 151-163
11. Manoj Aswar et.al, Anthelmintic activity of *Ficusbenghalensis*, International Journal of Green Pharmacy, July-September 2008,170-172
12. Singh, D., et.al Traditional uses, phytochemistry and pharmacology of *Ficusreligiosa*: A review. Journal of Ethnopharmacology, 2011, *134(3), 565-583.*
13. Chandrasekar S.B et al,phytopharmacology of *Ficusreligiosa*,Pharmacogn Rev.2010 Jul-Dec;4(8);195-199
14. Faiyaz Ahmed and AsnaUrooj , Traditional uses, medicinal properties, and phytopharmacology of *Ficusracemosa*: A review, Pharmaceutical Biology, 2010; 48(6): 672–681.
15. Yueh-HsiungKuo, Yen-Cheng Li,Constituents of the Bark of *Ficusmicrocarpa* Lf,Journal of the Chinese Chemical Society, 24 Sept 2013.
16. Rivastava, S., & Chandra, D. (2013). Pharmacological potentials of *Syzygiumcumini*: a review. Journal of the Science of Food and Agriculture, 93(9)
17. Kumar, RV., Augusti, KT. Hypoglycemic effect of a leucocyanidin derivative isolated from the bark of *Ficusbenghalensis* Linn. Indian Journal of Biochemistry and Biophysics1989; 26: 400–404
18. Patel DK Et.al,An overview on anti-diabetic medicinal plants having insulin mimetic property, Asian Pacific Journal of Tropical Biomedicine 2012,320-330.
19. MahalingamGayathri and Krishnan Kannabiran, Anti-diabetic And Ameliorative Potential Of *Ficusbengalensis* bark extract in Streptozotocin Induced Diabetic Rats, Indian Journal of Clinical Biochemistry, 2008 / 23 (4) 394-400
20. Shukla R, Anand K, Prabhu K, Murthy PS. Hypoglycaemic effect of the water extract of *Ficusbenghalensis* in alloxan recovered, mildly diabetic and severely diabetic rabbits. International Journal of Diabetes in Developing Countries 1994; 14:78-81.
21. Singh P, Singh VK, Singh AK. Molecular docking analysis of candidate compoundsderived from medicinal plants with type 2 diabetes mellitus targets. Bioinformation. 2019 Mar 15; 15(3):179-188.
22. Singh, Rakesh& Mehta, Shikha&Jaiswal, Dolly &Rai, Prashant&Watal, Geeta. (2009). Anti-diabetic effect of *Ficusbenghalensis* aerial roots in experimental animals. Journal of ethnopharmacology. 123. 110-4.
23. Singh AB, Yadav DK, Maurya R, Srivastava AK. Antihyperglycaemic activity of alpha-amyrin acetate in rats and db/db mice. Nat Prod Res. 2009; 23(9):876-82.
24. Pandit, R., Phadke, A., &Jagtap, A. (2010). Anti-diabetic effect of *Ficusreligiosa* extract in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology, 128(2), 462–466
25. Ambika, S.H., Rao, M.R.R., 1967. Studies on a phytosteroin from the bark of *Ficusreligiosa*,Indian Journal of Pharmacy 29, 91–94.
26. Kirana H, Agrawal SS, Srinivasan BP. Aqueous extract of *Ficus religiosa*linn. reduces oxidative stress in experimentally induced type 2 diabetic rats. Indian J Exp Biol. 2009 Oct; 47(10):822-6.
27. Brahmachari HD, Augusti KT. Orally effective hypoglycaemic agents from plants. J Pharm Pharmacol. 1962 Apr; 14:254-5.
28. Keshari AK, Kumar G, Kushwaha PS, Bhardwaj M, Kumar P, Rawat A, Kumar D, Prakash A, Ghosh B, Saha S. Isolated flavonoids from *Ficus racemosa* stem bark possess anti-diabetic, hypolipidemic and protective effects in albino Wistar rats. J Ethnopharmacol. 2016 Apr 2; 181:252-62.
29. Joshi, H., Vaishnav, D., Sanghvi, G., Rabadia, S., Airao, V., Sharma, T., Parmar, S., Sheth, N., 2016. *Ficusrecemosa* bark extract attenuates diabetic complications and oxidative stress in STZ-induced diabetic rats. Pharm. Biol. 54, 1586–1595
30. Sophia, D., Manoharan, S., 2007. Hypolipidemic activities of *Ficusracemosa*Linn.barkin alloxan induced diabetic rats. Afr. J. Tradit. Complement. Altern. Med. 4, 279–288.

31. Gul-e-Rana, Karim S, Khurhsid R, Saeed-ul-Hassan S, Tariq I, Sultana M, Rashid AJ, Shah SH, Murtaza G. Hypoglycemic activity of *Ficus racemosa* bark in combination with oral hypoglycemic drug in diabetic human. Acta Pol Pharm. 2013 Nov-Dec;70(6):1045-9
32. Ravichandiran, V., Nirmala, S., Ahamed, K.F.H.N., 2012. Protective effect of tannins from *Ficus racemosa* in hypercholesterolemia and diabetes induced vascular tissue damage in rats. Asian Pac. J. Trop. Med. 5, 367–373
33. BhaskaraRao, R., Murugesan, T., Sinha, S., Saha, B.P., Pal, M., Mandal, S.C., 2002. Glucose lowering efficacy of *Ficusracemosa* bark extract in normal and alloxan diabetic rats. Phytother. Res. 16, 590–592
34. Ahmed F, Urooj A. Effect of *Ficusracemosa* stem bark on the activities of carbohydrate hydrolyzing enzymes: an in vitro study. Pharm Biol. 2010 May; 48(5):518-23.
35. Jahan, I.A., Nahar, M., Mosihuzzaman, M., Rokeya, B., Ali, L., Khan, A.K.A., Makhmur, T., Choudhary, M.I., 2009. Hypoglycaemic and antioxidant activities of *Ficus racemosa* Linn. Fruits Nat. Prod. Res. 23, 399–408
36. Asok Kumar, M. Uma Maheswari , A.T. Sivashanmugam , V. Subhadra Devi , N.V. Prasanth and T.K. Ravi , 2007. Hypoglycemic Effect of *Ficusmicrocarpa* Leaves (Chinese Banyan) on Alloxan-induced Diabetic Rats. Journal of Biological Sciences, 7: 321-326
37. Saravanan G and Leelavinothan P, Effects of *Syzygiumcumini* bark on blood glucose, plasma insulin and c-peptide in streptozotocin induced diabetic rats. Int J EndocrinolMetab 4: 96-105 (2006).
38. Schossler DRC, Mazzanti CM, Luz SCA, Filappi A, Prestes D, Silveira AF, et. al., *Syzygiumcumini* and the regeneration of insulin positive cells from the pancreatic duct. Braz J Vet Res AnimSci 41: 236-239 (2004)
39. A. Kumar et al, Anti-diabetic activity of *Syzygiumcumini* and its isolated compound against streptozotocin-induced diabetic rats, Journal of Medicinal Plants Research Vol. 2(9), pp. 246-249, September, 2008.
40. Chaturvedi A, Bhawani G, Agarwal PK, Goel S, Singh A and Goel RK, Anti-diabetic and anti-ulcer effects of extract of *Eugenia jambolana* seed in mild diabetic rats: study on gastric mucosal offensive acid-pepsin secretion. Indian J PhysiolPharmacol 53(2): 137- 146 (2009).
41. Prince PSM, Kamalakkannan N and MenonVP, Anti-diabetic and anti-hyperlipidaemic effect of alcoholic *Syzigiumcumini* seeds in alloxan induced diabetic albino rats. J Ethnopharmacol 91: 209-213 (2004).
42. Prince PSM, Menon VP and Pari L, Hypoglycaemic activity of *Syzigiumcuminis*eeds:effect on lipid peroxidation in alloxan diabetic rats. J Ethnopharmacol 61: 1-7 (1998).

Table no: 1. Traditional usage of Siddha preparation of *Panchathuvarpi* for Diabetes

Siddha (Tamil Name)	Scientific name	Parts used	Traditional usage form for Diabetes
Aal	***Ficus benghalensis***	Bark, leaves, fruit, aerial root, latex, flower	• Bark – *Ooralneer* (cold infusion)[2] • Leaves-*AalilaiSaambal* (Ash of mature leaves)[3] • Latex(external application for Diabetic ulcers and carbuncles)[4] • As an ingredient in Decoction for external wash of Diabetic ulcers[4]
Arasu	***Ficus religiosa***	Barks, leaves, root, latex, seed, flower	• Whole plant is indicated for diabetes[5] • As an ingredient in Decoction for external wash of Diabetic ulcers[4]
Atti	***Ficus racemosa***	Bark, fruit, latex, toddy	• Fruit-*Manappagu*(Syrup) • Latex (external application for Diabetic ulcers and carbuncles)[2] • Latex -External application for diabetic carbuncles)[6] • *Attiyathikashayam*[7] • As an ingredient in Decoction for external wash of Diabetic ulcers[4]

Itti	***Ficus microcarpa***	Bark,fruit	• As an ingredient in Decoction for external wash of Diabetic ulcers[4]
Naaval	***Syzygium cumini***	Bark,seed,fruit,root	• Bark-*Kashayam*(decoction) • Root–*Ooralneer*(cold infusion) • Seed- *Choornam*(powder) • Fruit–*Aristam*(fermented form)[2] • Seed –as an ingredient inDecoction for diabetes • *Naval podi*[6] • Bark -As an ingredient in *Maruthampattaikashayam*(cardiac tonic) • Bark -As an ingredient in *Aavaraiyathikashayam*[7]

Table no: 2. Phytoconstituents of *Panchathuvarpi*

Name of the Herb	Parts Used	Phytoconstituents
Aal (Ficus benghalensis)	Bark	• Derivatives of Anthocyanidin;3',5 dimethyl ether of leucocyanidin 3-0-alpha-D galactosylcellobioside,5,7-dimethyl ether of leucopelargonidin 3-0-alpha-L rhamnoside,3',5,7-trimethyl ether of delphinidin-3-O-αL-rhamnoside,3',5,7-trimethylether of leucocyanidin. • Phytosterols;α-amyrin acetate ,lupeol, Lanostadienylglucosylcetoleate, benghalensisteroic acid acetate[10] • Meso-inositol, Sterol; β Sitosterol-α-d-glucose 20- tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one[11]
	Leaves	• Sterols ;β-sitosterol, • Flavonoids ; genistein ,Catechin , • Flavonols; Quercetin-3-galactoside, rutin, Leucoanthocyanidins; Leucocyanidin, • Triterpenoids;Friedelin[10] • Crude protein 9.63%, crude fibres 26.84%, CaO-2.53%, and Phosphorus-0.4%.
	Latex	• Caoutchoue (2.4%), Albumin, Cerin, Resin, Sugar and Malic acid.[11]
Arasu (Ficus religiosa)	Bark	• 8.7% of total tannin, • Lanosterol -Sitosterol and its glucoside (-sitosteryl-d-glucoside) and Stigmasterol, Vitamin K1
	Fruit	• Asparagine and tyrosine, alanine, aspartic acid, glycine, threonine, norleucine and norvaline. • Flavonols like, myricetin ,quercetin and kaempferol, condensed tannins also in the immature fruits. • Volatile components • Fibers such as hemicellulose, cellulose ,lignin and pectin , serotonin.
	Leaves	• Phytosterols like, campesterol, stigmasterol, sitosterol and 28-isofucosterol. • 1.5% of total tannin content, which comprises tannic acid and condensed tannins • minerals like, calcium, phosphorous, iron, copper, manganese, magnesium, zinc, potassium and sodium[12]

	Seeds	• Phytosterolin, β-sitosterol, and its glycoside, albuminoids. • carbohydrate, fatty matter, ,caoutchoue 0.7–5.1%.[13]
Atti (Ficus racemosa)	Stem bark	• steroids, alkaloids, tannins, gluanol acetate, leucocyanidin-3-O-β-D-glucopyrancoside, leucopelargonidin-3-O-β-D-glucopyranoside, leucopelargonidin-3-O-α-L-rhamnopyranoside, cerylbehenate, lupeol acetate, α-amyrin acetate ,lupeol, friedelin, behanate, β-sitosterol, β-sitosterol-D-glucoside , gluanol acetate and quercetin. Bergenin, racemosic acid, , β-sitosterol, β-amyrin, and lupeol acetate
	Fruits	• sterols, triterpenoids, flavonoids, glycosides, tannins, carbohydrate, β-sitosterol, gluanol acetate, hentriacontane, tiglic acid of taraxasterol, lupeol acetate, gallic acid, ellagic acid and α-amyrin acetate[14]
Itti (Ficus microcarpa)	Bark	• Oleanolic acid, betulinic acid, lupeol, catechin and β-sitosterol, gallic acid , phenolic acid, • Steroid;.ergostane and stigmastane • Triterpene compounds ;friedelane, ursane, oleanane, lupane, cycloartane, and taraxerane • Flavane ;catechi • Carotene like compound;4,5-Dihydroblumenol • Steroids; p-sitosterone, P-sitostenone, p-sitosterol, stigmasterol , 6 p-hydroxystigmast-4-en-3-one , ergosterol peroxide ,6'-(P-sitosteryl-3-0-P-glucopyranosidylhexadecanoate and P-sitosteryl-3-O-P-glucopyranoside. • coumarin ;marmesin[15]
Naaval (Syzygium cumini)	Stem Bark	• Betulinic acid, β-sitosterol, friedelin, epi-friedelanol, ester of epi-friedelanol, β-sitosterol-D-glucoside, kaempferol-3- glucoside, quercetin, myricetin, astragalin and gallic acid
	Fruit	• Malic acid , oxalic acid, gallic acid , tannins, cyanidindi glycosides
	Seed	• A glucosidejamboline, • Phenolics such as ellagic acid, gallic acid, caffeic and ferulic acids, • Flavonoids such as rutin, quercetin • 9 β-sitosterol, chlorophyll, fat, resin, albumen, tannins[16]

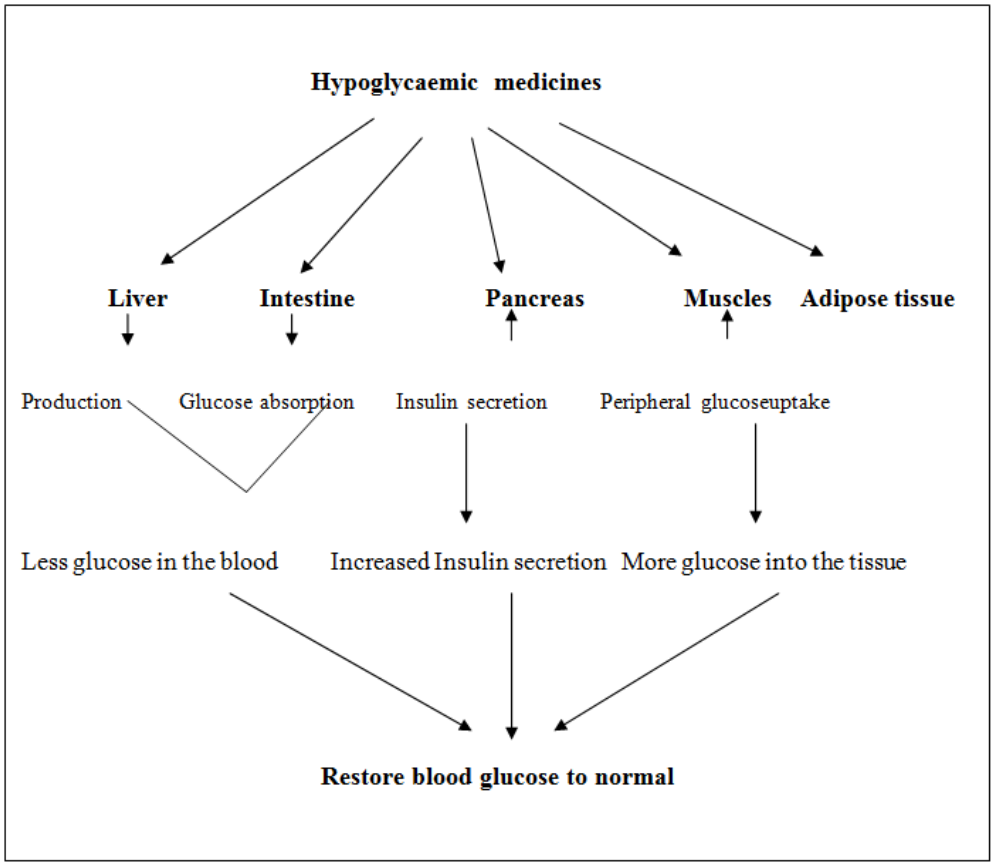

Fig.1. Mechanism of Anti-diabetic drug

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RESEARCH ARTICLE

Metagenome and Culture Based Study of Commercial Potato Chips

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ABSTRACT

The study was performed out to investigate the microbiological quality of commercially available potato chips available in the Indian market. In the current study, bacterial counts of 4 flavours of potato chips were analysed using biochemical and metagenomic approaches. Among the samples analysed majority were found to contain cyanobacteria. The present study revealed that potato chips were highly contaminated with cyanobacteria and poses a threat for human consumption due to potential production of cyanotoxins.

Keywords: biochemical, bacteria, DNA, metagenome, potato chips

INTRODUCTION

Potato chips are thinly cut potatoes that have been baked or fried and lightly salted or seasoned. It is a very popular dry snack items all over the world which are available in most bakeries or grocery shops. Commercially available potato chips are blended in with various sorts of flavorings and fixings including spices, flavors, cheeses, regular or artificial flavors and added substances and making it crispier and savoury. Most potato chips contain high levels of sodium and salt this linked with high blood pressure and heart problems [1]. An important concern is the presence of acrylamide that is a potential carcinogen that is formed during the frying process [2,3]. Certain processes such as elevated temperature and presence of starch in food items favour acrylamide production via the Maillard reaction [4].

As chips are prepared as ready to eat food product, they are susceptible for microbial contamination that can arise during the storage or transportation process. Some microbes namely *E. coli, Pseudomonas sp , Listeria monocytogenes , Klebsiella sp, Vibrio sp and Staphylococcus aureus* have been reported to cause spoilage [5]. Besides, presence of fungus can lead to food intoxication due to toxin production. The present study was performed to enumerate bacterial counts of commercially available chips and determine their overall quality for assessing the public health risks.

MATERIALS AND METHODS

Sample collection

A total of 5 brands of chips (Lays brand) were procured and bacterial isolation were performed in sterile conditions. Gram staining kit, nutrient agar, and reagents for microbiological tests were purchased from Himedia labs, India. Metagenomic DNA isolation and sequencing were performed at RTL Genomics Inc, USA [6]. Data analysis, sequence clustering and taxonomic identification were performed using bioinformatics tools [7,8].

Isolation of bacteria

The streak plate technique was used for isolation of bacterial strains from chips samples. The bacteria were isolated by spread plate method on nutrient agar medium, incubated at 37°C for 24 h to obtain colonies. The individual colonies were picked upon the basis of their macroscopic characters such as size, shape, surface appearance, texture, and color. These colonies were subjected to repeated streaking on nutrient agar plates/slants.

Morphological characterization of isolated bacteria

Colony and cell morphology based on their color, shape, margin, elevation, surface, and arrangement of bacteria were studied.

Microscopic observation

Simple and Gram staining of the isolates were performed according to standard protocols.

Biochemical characterization of isolated bacteria

Catalase test

Catalase annihilates the bactericidal impacts of hydrogen peroxide. Catalase facilitates the breakdown of H_2O_2 into H_2O and O_2. This is due to the fast development of air pockets. The culture sample was taken and kept in the slide. The hydrogen peroxide solution was added drop by drop. The air bubbles were observed in the positive samples.

MR-VP test

MR-VP medium was autoclaved. The MR-VP medium was introduced with the microorganisms from the potato chips, incubated and Methyl red was added [10]. The development of red shading showed positive outcome for Methyl Red test for the microorganisms of the potato chips. MR-VP medium was autoclaved. The microscopic organisms from the chips were added into the medium. Barrit's reagent was introduced.

Citrate utilization test

The simmon's citrate agar medium was autoclaved. The simmon's citrate agar medium was made as agar inclines. The confined microorganisms of the potato chips were introduced. The test tubes were noticed for shading change.

Urease test

Christens Urea Agar media agar slants were prepared under aseptic conditions. A loopful of the sample was inoculated and the slant was incubated at 37 ºC for 24 hrs. An uninoculated slant served as control and any colour change in the test samples were recorded.

Antibiotic sensitivity test

A sterile cotton swab was used to collect the sample and it was uniformly spread on the surface of the petri plate to obtain an even lawn culture. Under aseptic conditions, antibiotic discs were placed. The petri plates were incubated for 24 hours at 37ºC.

RESULTS AND DISCUSSION

Morphology

Bacterial colonies were isolated from the chips samples and Gram staining results indicated Gram-positive rod-shaped bacteria present in clumps (Fig 1). Biochemical testing (Table 1) revealed that the bacteria isolated from all the four different flavored chips packing were catalase positive and could ferment glucose to produce acid (Fig 2). The isolates showed positive results for amylase production but negative for Voges Proskauer and urease and indole tests respectively (Fig 3). Citrate and TSI tests yielded positive results and revealed the utilization of citrate with formation of alkaline end products. Absence of black precipitate in the slant revealed that the bacteria were unable to produce H_2S (Fig 4).

Metagenomic analysis

Taxonomic studies revealed the presence of abundant bacteria trimmed kingdom and ranked first (99 %) and the rest were no hit [Table 2]. Among trimmed phylum, cyanobacteria accounted to the vast majority of microbes (99.11 %) while there was no hit to 0.81 % of the metagenomic sequences. Among trimmed order and family Oscillatoriales accounted to the majority (99.11 %) while rhizobiales were present at an extremely low proportion (Table 3). Amongst the genera, *Halospirulina* sp was found in a larger proportion when compared to *Bradyrhizobium* sp. (Table 4)

CONCLUSION

The results of biochemical tests revealed the presence of aerobic microbes that could have a possible symbiotic relationship with potatoes. Similarly, presence of cyanobacterial strains could possibly indicate contamination in chips and can pose as a hazard for human consumption.

REFERENCES

1. Hashemian M, Murphy G, Etemadi A, Liao LM, Dawsey SM, Malekzadeh R, et al. (2019) Potato consumption and the risk of overall and cause specific mortality in the NIH-AARP study. PLoS ONE 14(5): e0216348
2. Mesias M, Delgado-Andrade C, Holgado F, Morales FJ 2019 Acrylamide content in French fries prepared in food service establishments LWT-Food Science Technology, 100, 83-91.
3. Martinez E, Rodriguez G A, Mondragon A C, Lorenzo J M, Santos E M (2019) Influence of potato crisps processing parameters on acrylamide formation and bioaccesibility. Molecules 24 , 3827
4. Delgado-Andrade, C. 2014. Maillard reaction products: some considerations on their health effects. *Clin. Chem. Lab. Med.* 52:53–60
5. Tamberkar DH, Kulkarni RV, Shirsat SD, Bhadang DG (2011). Bacteriological quality of street vended food panipuki. Bioscience discovery, 12(3): 350-354
6. Huse M et al. 2007 Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biology 8 (77) 1.
7. Edgar R C 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10 (10) 996-998.
8. Haas B J et al. 2011. Chimeric 16 S rRNA sequence formation and detection in Sanger and 454 pyrosequenced PCR amplicons. Genome Research 21 (3) 494-504

Table 1. Summary of biochemical test results of the microbes isolated from chips

S.No	Name of the assay / test	Result
1	Simple staining	Rod shaped
2	Gram staining	Gram positive
3	Catalase test	Positive
4	Methyl red test	Positive
5	Voges – Proskauer test	Negative
6	Citrate utilization test	Positive
7	Urease test	Negative
8	TSI test	Positive
9	Starch hydrolysis test	Positive
10	Indole test	Negative
11	Antibiotic susceptibility test	Positive

Table 2. Taxonomic distribution of microbial populations

Kingdom	Trimmed Taxa Percentage
Bacteria	99.18
No Hit	0.81

Table 3. Order percentage distribution of microbial populations

Order	Trimmed Order Percentage
Oscillatoriales	99.11
Rhizobiales	0.07
No Hit	0.81

Table 4. Percentage distribution of microbial genera

Order	Trimmed Genera Percentage
Halospirulina	99.11
Bradyrhizobium	0.07
No Hit	0.81

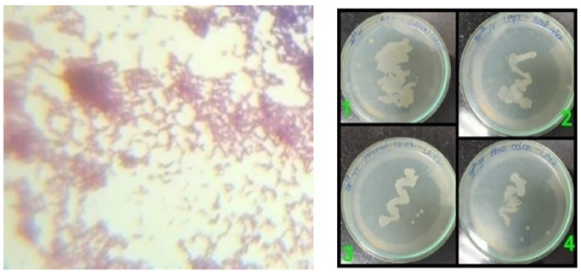

Fig. 1. Isolation and Gram staining of bacteria from chips samples

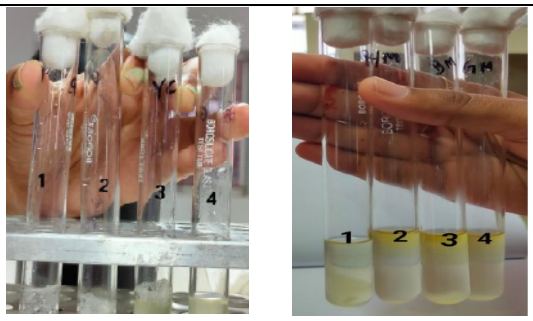

Fig. 2. Catalase and Methyl red tests for bacterial isolates

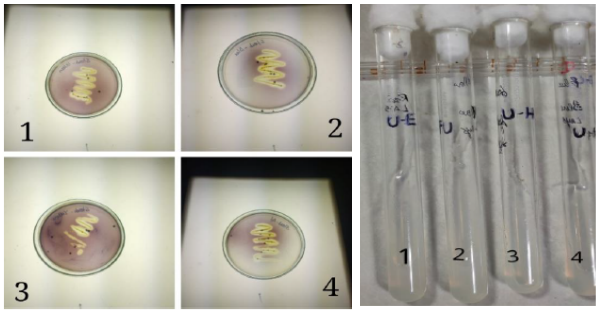

Fig. 3. Starch hydrolysis and urease tests for bacterial isolates

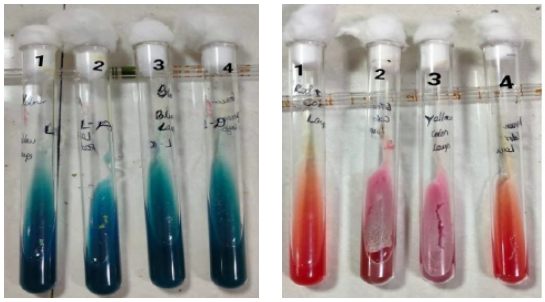

Fig. 4. Citrate utilization and and TSI tests for bacterial isolates

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RESEARCH ARTICLE

Hepatoprotective Activity of *Eichhornia crassipes* Flowers against Carbon Tetrachloride Induced Liver Damage in Wistar Rats

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ABSTRACT

The objective of the present study was to assess the hepatoprotective potential of ethanol extract of *Eichhornia crassipes* flowers against carbon tetrachloride-induced hepatocellular damage. The extract was subjected to preliminary phytochemical examination and quantitative estimation of bioactive compounds. The *in-vitro* antioxidant activity was evaluated by DPPH radical scavenging assay, lipid peroxidation inhibitory activity, Nitric oxide scavenging activity, Ferric reducing antioxidant capacity and Cupric ions (Cu^{2+}) reducing antioxidant capacity**.** The *in-vivo* hepatoprotective potential was evaluated in wistar rats by administration of extract in doses 200 mg/kg and 400 mg/kg for 7 days followed by intraperitoneal injection of single dose of 1 ml/kg CCl_4 in olive oil. The preliminary phytochemical screening showed the presence of phenolics, tannins and flavonoids. The quantitative estimation showed the presence of phenolic, tannins and flavonoids in the extract. The *in-vitro* antioxidant assay results showed a dose dependent free radical scavenging and anti-oxidant activity. The hepatoprotective potential of extract was evidenced by a significant decrease in serum marker enzymes and a significant increase in serum protein levels. In liver tissue, a significant decrease in lipid peroxidation and a significant increase in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glutathione-S-transferase (GST) was noted. Gross examination and histological observations of liver tissue too correlated with the biochemical observations. These findings suggest that *Eichhornia crassipes* flower extract possess hepatoprotective property against CCl_4 induced liver damage which might be due to stabilization and increase in all the components of antioxidant system attributed to antioxidant and free radical scavenging activity.

Keywords: *Eichhornia crassipes* flowers; Carbon tetrachloride; Hepatotoxicity; Anti-oxidant; Hepatoprotective.

INTRODUCTION

Liver plays major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment of its functions may lead to many implications on one's health [1]. Management of liver diseases is still a challenge to the modern medicine. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [2]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in ayurveda recommended for the treatment of liver disorders [3]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systemic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Traditionally, many herbal medicines and medicinal plants have been used for treatment of complicated diseases/disorders. Among the herbal resources is *Eichhornia crassipes* (Mart.) Solms belongs to family Pontederiaceae. *Eichhornia crassipes* (Pontederiaceae), commonly known as water hyacinth, is one of the aquatic plants that have attracted the most scientific interest in the last decade. Originally from South America, its ornamental appeal led to its introduction into Africa, Asia, the South Pacific, North America and Europe, where it has became invasive [4]. Plants can grow up to 3 feet in height above the surface of the water. The leaves are oval to elliptical, thick, up to 6 inch wide and waxy with spongy petioles, curved inwards at the edges. Flowers are blue-purple on upright spikes. Each flower has six petals with the uppermost having a yellowish patch [5].*Eichhornia crassipes* is capable of bio-concentrating toxic metals such as Cr, Cu, Co, Ni, Pb, Cd and as in its root system [6]. It is also reported to possess valuable phytochemicals which are of medicinal importance [7].The fresh juice of this weed is used by tribes to treat wounds, to ease swelling, burning and to stop bleeding [8].*Eichhornia crassipes* leaves have been reported to possess a potential antibacterial, antifungal, antiparasitic, antiviral and anticancer activity [9].Scientifically there are no valid reports available on hepatoprotective potential of *Eichhornia crassipes* flowers. Hence, the present study was aimed to investigate the anti-oxidant and hepatoprotective potential of *Eichhornia crassipes* flower extract.

MATERIALS AND METHODS

Plant Material

The fresh flowers of *Eichhornia crassipes* were collected in the month of June from Bhavani River, Tamilnadu, India. The plant material was taxonomically identified, confirmed and authenticated by Dr. A. Balasubramanian, Executive Director and Former Siddha Research Consultant (AYUSH), ABS Herbal gardens, Salem and the authentication was retained in our laboratory for further reference. The collected flowers were shade dried and the dried materials were crushed to coarse powder with mechanical grinder. The powder was stored in an airtight container for extraction.

Extraction

The powdered flowers of *Eichhornia crassipes* were subjected to hot continuous extraction using ethanol as a solvent material in Soxhlet apparatus for 72 hours. After extraction, the solvents were removed by distillation and evaporated under reduced pressure in a rotary evaporator to obtain crude extract of *Eichhornia crassipes* flowers. The collected extract was then transferred to a clean glass vessel and covered with a foil paper in which slits are made for evaporation of solvent traces. The dried extract thus obtained was stored in air tight glass container for further investigation.

Percentage yield and Phytochemical screening

The extract obtained was subjected to percentage yield and preliminary phytochemical screening [10].

Quantitative estimation of bioactive compounds in ethanol extract of *Eichhornia crassipes* flowers

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu method as described by Slinkard and Singleton [11] and Singleton & Rossi [12]. The total tannin content was determined by modified method of Polshelttiwar*et al.,*[13]. Flavonoid content was measured using aluminum chloride colorimetric method as described by Chang [14] The antioxidant activities of various solvent extracts were evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al.,* [15].

Determination of free radical scavenging activity

DPPH radical scavenging activity was determined spectrophotometrically using the method of Blois [16]. The lipid peroxidation inhibitory activity of the flower extract was determined according to the method of Duh and Yen [17]. The nitric oxide scavenging activity of the flower extract was determined according to the method of Green *et al.,* [18]. According to the method described by Oyaizu [19], ferric reducing powers of extracts were determined. By the method of Apak*et al.,* [20], the cupric reducing antioxidant capacity (CUPRAC) was determined.

Hepatoprotective activityof *Eichhornia crassipes* flowers

Animals

Healthy, young adult female albino wistar rats (150-200 g) were used for the study. The animals were obtained from Mass Biotech (2084/PO/Bt/S/19/CPCSEA), Chengalpet, Tamilnadu and were housed in polypropylene cages. The animals were maintained under standard laboratory conditions (25° ± 2°C; 12 hr light and dark cycle). The animals were fed with standard diet and water *adlibitum*. Ethical clearance (for handling of animals and the procedures used in study) was obtained from the Institutional Animal Ethical Committee (JKKMMRAFCP/IAEC/2019/008) before performing the study on animals.

Acute oral toxicity of ethanol extract of *Eichhornia crassipes* flowers

Acute oral toxicity of ethanol extract of *Eichhornia crassipes* flowers was performed in accordance to OECD Test Guidelines 425 [21].

Carbon tetrachloride-induced hepatotoxicity (Acute model)

Experimental design

Healthy male albino wistar rats (6-8 weeks) weighing 130-150g were used for the study. Randomization in selection of animals for grouping was carried out to avoid statistical difference in the body weight of animals. The experimental animals were divided into five groups, each group comprising of six animals [22]. Group I animals served as normal control rats received vehicle 0.5% CMC 1ml/kg/day orally for 7 days.Group II served as a negative control received vehicle 0.5% CMC 1ml/kg/day orally for 7 days.Group III animals received ethanol extract of *Eichhornia crassipes* flowers 200 mg/kg in 0.5% CMC orally for 7 days.Group IV animals received ethanol extract of *Eichhornia crassipes* flowers 400 mg/kg in 0.5% CMC orally for 7 days.Group V animals served as a positive control group received silymarin 100 mg/kg as a reference in 0.5% CMC orally for 7 days.On day 7, 1 hour after the last dose administration, the animals in groups II–V were administered intraperitoneally (i.p) with 1 ml/kg CCl_4 in olive oil (1:1). After 48 h of CCl_4 administration, the blood samples were collected via retro-orbital plexus and the clear serum was separated by centrifugation at 2500 rpm for 15 min at 4°C. The separated serum was subjected to biochemical analysis.

Biochemical analysis

Serum transaminases (AST and ALT) were determined by the method of Reitman and Frankel [23]. The activity of serum alkaline phosphatase (ALP) was estimated by the method of Kind and King [24]. Serum bilirubin (SB) and total protein (TB) levels were estimated by the methods of Malloy and Evelyn [25] and Wooten [26] respectively.

Morphological examination of liver

The animals were euthanized by carbon dioxide inhalation and dissected. The liver was carefully excised, washed in ice cold saline, blotted to dryness and examined for any deductable changes.

Measurements of liver weight

To reduce the differences from individual body weights, the relative liver weights [27] (as a percentage of body weights) were calculated from the following formula:

$$\text{Relative liver weights (\% of body weight)} = \frac{\text{absolute liver wet-weights}}{\text{body weight at sacrifice}} \times 100$$

Measurement of liver lipid peroxidation and anti-oxidant defense system

For the estimation of non-enzymatic and enzymatic antioxidants, tissues were minced and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged for 10 min and the resulting supernatant was used for enzyme assays. The level of thiobarbituric acid reactive substances (TBARS) in the liver was measured by the method of Ohkawa*et al.,* [28] as a marker for lipid peroxidation. Superoxide dismutase (SOD) activity was assessed according to the procedure of Kakkar*et al.,*[29]. Catalase (CAT) activity was measured according to the procedure of Sinha [21].GPx activity was measured by the method of Rotruck*et al.,*[30].Glutathione-S-transferase (GST) activity was assessed according to the protocol of Habig*et al.,*[31]**.** Glutathione reductase (GR) activity was determined according to the method described by Horn and Burns [33]. Reduced glutathione(GSH) was estimated by the method of Ellman [34].

Histopathological examination

A portion of liver tissue was subjected to histopathological examination. A portion is fixed in 10% formalin and embedded in molten paraffin wax and were ultra-sectioned (5-6 μm thickness). The tissues were stained with hematoxylin and eosin (H & E) and were examined under light microscope for histopathological changes.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). The results were analysed for statistical significance by one way ANOVA followed by dunnett's test (Graphpad Software Inc,La Jolla, CA. Trial version). The criterion for statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Percentage yield and Preliminary phytochemical studies

The percentage yield of extract obtained from powdered *Eichhornia crassipes* flowers using ethanol as solvent was 7.25 % w/w. Preliminary qualitative investigation performed in the ethanol extract of *Eichhornia crassipes* flowers revealed the presence of major phytoconstituents alkaloids, phenolic compounds, tannins, flavonoids, sterols, terpenoids, glycosides, carbohydrates, proteins and aminoacids.

Quantitative estimation of bioactive compounds

The total phenolic content in EEEC was found to be 237.60 ± 2.36 µg GAE equivalents per mg of dry extract. The total tannin content in EEEC was found to be 365.35 ± 2.85 μg of TAE equivalent per mg of dry extract. The total flavanoid content in EEEC was found to be 247.60 ± 2.45 μg of quercetin equivalent per mg of extract.

Total antioxidant capacity

The total antioxidant activity of EEEC flowers was evaluated by phosphomolybdenum method and the results were expressed as ascorbic acid equivalents. The ethanol extract showed a potent antioxidant activity with an antioxidant capacity of 441 ± 1.32 µg ascorbic acid equivalents per mg of dry extract.

Free radical Scavenging activity

The ethanol extract showed a significant dose-dependent inhibition of DPPH activity with a 50 % inhibition (IC_{50}) at a concentration of 79 ± 0.20 µg/ml which was comparable to reference standard ascorbic acid with IC_{50} value of 15.52 ± 0.18 µg/ml. It was observed that EEEC flowers showed a weak inhibitory effect on ultrasound induced lipid peroxidation in liposome prepared from egg lecithin. The IC_{50} value of EEEC was found to be above 1000 µg/ml, whereas standard drug tocopherol showed an IC_{50} value of 2.13 ± 0.11 µg/ml. The EEEC flowers showed nitric oxide scavenging activity by reducing the amount of nitrite generated from the decomposition of sodium nitroprusside *in-vitro*. The IC_{50} value of EEEC was found 389 ± 2.24 µg/ml and the IC_{50}value of standard compound quercetin was found to be 12.0 ± 0.16 µg/ml respectively. It was observed that the reducing ability of the extracts increased with the concentration.

CUPRAC assay

EEEC flowers showed CUPRAC reducing capacity of 159.68 ± 2.50 µg of Trolox/mg of extract.

Acute oral toxicity study

Acute oral toxicity study was carried out as per OECD guideline 425. From the limit test results it was observed that the extract of *Eichhornia crassipes* flowers was safe up to a dose level of 2000 mg/kg.

Hepatoprotective activity against carbon tetrachloride-induced hepatotoxicity

Gross morphology of liver

The appearance of liver in control group I rats were normal and there were no macroscopically detectable changes in liver. The Liver of group II rats intoxicated with carbon tetrachloride was pale in appearance with enlarged and swelled (inflammation) morphological appearance. The liver of group III animals treated with *Eichhornia crassipes* flower extract 200 mg/kg and CCl_4 intoxicated showed mild reduction in morphological changes caused by CCl_4 and the liver of group IV animals treated with *Eichhornia crassipes* flower extract 400 mg/kg and CCl_4 intoxicated showed mild swelling and discoloration. The morphology of liver in group V animals treated with silymarin 100 mg/kg and CCl_4 intoxicated was normal in appearance which was similar to control animals.

Relative liver weight

The results were expressed as g liver/100 g body wt. The relative liver weight of group II CCl_4 intoxicated untreated animals were (4.15 ± 0.05 g%)significantly increased ($p<0.0001$) compared to Group I control animals (3.39 ± 0.01 g%). A significant decrease in relative liver weight was noted in group III, group IV and group V animals treated with extract and CCl_4 intoxicated compared to CCl_4 intoxicated untreated animals. The relative liver weight of group treated with extract 200 mg/kg was 3.69 ± 0.05 g % ($p<0.001$) and 400 mg/kg ($p<0.0001$) was 3.49 ± 0.03 g % ($p<0.0001$). The relative liver weight of group treated with silymarin 100 mg/kg was 3.45 ± 0.07 g %.

Effect on biochemical parameters

The animals treated with *Eichhornia crassipes* flower extract 200 mg/kg and 400 mg/kg with CCl_4 intoxicated showed a significant decrease ($P<0.0001$) in AST, ALT, ALP and total bilirubin levels. The animals treated with silymarin 100 mg/kg and CCl_4 intoxicated showed significant decrease ($P<0.0001$) in AST, ALT, ALP and total bilirubin levels. The animals treated with *Eichhornia crassipes* flower extract 200 mg/kg and 400 mg/kg showed significant increase in the total protein, albumin and globulin levels compared to CCl_4 intoxicated untreated animals. The animals treated with silymarin 100 mg/kg and CCl_4 intoxicated showed significant increase in total protein, albumin and globulin levels.

Effect on liver enzymes

Administration of *Eichhornia crassipes* flower extract and CCl_4 intoxicated showed a significant reduction in LPO as evidenced by a significant fall in MDA levels. The animals treated with silymarin 100 mg/kg and CCl_4 intoxicated showed significant fall ($p<0.0001$) in MDA levels. The activities and the levels of antioxidants in liver of control and experimental animals and the results were expressed as U/mg of protein. A significant decrease ($p<0.001$) in activities of SOD, catalase, GSH-Px,GR, GST and GSH were noted in group II CCl_4 intoxicated untreated animals compared to control animals. However, administration of *Eichhornia crassipes* flower extract at doses 200 mg/kg and 400 mg/kg significantly increased the levels of SOD, catalase, GSH-Px, GR, GST and GSH levels.

Histopathology

In histopathological studies showed the control group I animals revealed normal architecture of liver. Group II CCl_4 intoxicated animals showed altered lobular structure with parenchymal necrosis, hepatitis and extravasatedRBCs. Central vein congestion and sinusoid dilation were observed. Group III animals treated with *Eichhornia crassipes* flower extract 200 mg/kg and CCl_4 intoxicated showed altered lobular structure with mild necrosis. Cytoplasmic vacuolation and periportal inflammation was noted. Group IV animals treated with *Eichhornia crassipes* flower extract 400 mg/kg and CCl_4 intoxicated showedaltered lobular structure with mild necrosis and interface hepatitis. Portal triad showed mild periportal inflammation. Group V animals treated with silymarin 100 mg/kg and CCl_4 intoxicated showed normal lobular architecture with mild central vein dilation and congestion. Individual hepatocytes showed mild cytoplasmic vacuolation and binucleation.

DISCUSSION

Based on the widespread use of *Eichhornia crassipes*in traditional medicine, in the present study, an effort was made to ascertain the hepatoprotective potential of flowers of *Eichhornia crassipes*. Treatment with *Eichhornia crassipes* flower extract ameliorated the imbalance in liver enzymes and protein caused by CCl_4 in a dose dependent manner. This reversalof the enzyme levels by the extract is probably becauseof their membrane stabilizing activity which preventsleakage of intracellular enzymes. Restoration in the levels of lipid peroxidation and enzymatic antioxidant defense mechanisms upon treatment with *Eichhornia crassipes* flower extract might have resulted in the recoupment in the activities of the above antioxidant enzymes to normalcy. Thehistopathological findings are supported by the biochemical data of hepatoprotective abilities of *Eichhornia crassipes* flower extract. The observed hepatoprotective and antioxidant activities of *Eichhornia crassipes* flower extract are might be due to the presence of antioxidant phytochemicals like polyphenols including flavonoids, phenolic acids, tannins, stilbenes and lignans.

CONCLUSION

To conclude, the present investigation shows the protective effect of *Eichhornia crassipes* flowers against CCl_4 induced hepatotoxicity. However, the component responsible for the anti-oxidative activity remains unclear. Therefore, further studies are needed to isolate and identify the antioxidant compounds present in *Eichhornia crassipes* flower extract, and also to clarify the mechanisms of activity of the active compounds responsible for hepatoprotective activity.

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REFERENCES

1. Subramaniam S, Khan HBH, Elumalai N, Lakshmi SYS. Hepatoprotective effect of ethanolic extract of whole plant of *Andrographis paniculata*against CCl_4-induced hepatotoxicity in rats. Comp Clin Path 2015; 24:1–7.
2. Guntupalli M, Mohana R, Chandana V, Pushpangadan P, Shirwaikar A. Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. J. Ethnopharmacol 2006; 103(3): 484-490.
3. Chatterjee TK. Herbal Options inMedicinal plants with hepatoprotective properties 3rd ed. Calcutta: Books and Allied (P) Ltd; 2000, pp.135.
4. Jones RW, Hill JM, Coetzee JA, Hill MP. The contributions of biological control to reduced plant size and biomass of water hyacinth populations. Hydrobiologia 2018;807(1):377-388.
5. Gopal B. Water hyacinth. Elsevier Science Publishers, Amsterdam, New York, 1987, pp.477.
6. Ali M, Bhat AK, Dolkar T, Malik MA. Phytoremediation: A Plant-Based Technology.*International Journal* of *Current Microbiology* and *Applied* Sciences2018; **7**(3):766–777.
7. Lalitha P, Sripathi SK, Jayanthi P.Acute toxicity study of extracts of *Eichhornia crassipes* (mart.) Solms. Asian Journal of Pharmaceutical and Clinical Research2012; 5(4):59-61.
8. Oudhia P. Traditional medicinal knowledge about a noxious weed, jalakumbhi (*Eichhornia crassipes*), in Chhattisgarh (India), Aquaphyte Online, 2001.
9. Aboul-Enein AM, Al-Abd AM, Shalaby EA, Abul-Ela F, Nasr-Allah AA, Mahmoud AM, El-Shemy HA. *Eichhornia crassipes* (Mart) solms. From water parasite to potential medicinal remedy. Plant Signaling and Behavior 2011; 6(6):834-836.
10. Khandelwal KR and Kokate CK Pratical pharmacognosy, 4th ed, Vallabh Prakashan, New Delhi, 1995, pp.110.
11. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enol Viticult 1977; 28:49-55.
12. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult 1965; 16:144-158.
13. Polshettiwar SA, Ganjiwale RO, Wadher SJ, Yeole PG. Spectrophotometric estimation of total tannins in some ayurvedic eye drops. Indian J Pharma Sci2007; 69:574-576.
14. Chang C, Yang M, Wen H, Chen J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10:178–182.
15. Prieto P, Pineda M and Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E Anal Biochem1999;269:337-341.
16. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181:1199-1200.
17. Duh PD, Yen GH. Antioxidative activity of three herbal water extracts. Food chemistry 1997; 60:639-645.
18. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS and Tannenbaum SR. Analysis of nitrate, nitrite and (15N) nitrate in biological fluids. Anal Biochem 1982; 126:131-138.
19. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr1986;44:307–315.
20. Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method. J Agric Food Chem 2004; 52:7970-7981.
21. Organization for Economic Co-operation and Development-OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Publishing; 2000.
22. Zargar OA, Bashir R, Ganie SA, Masood A, Zargar MA, Hamid R.Hepatoprotective potential of *Elsholtziadensa* against acute and chronic models of liver damage in wistar rats. Drug Res (Stuttg) 2018; 68(10):567-575.
23. Reitman S, Frankel SA. Coloirmetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28:56-63.
24. Kind PRN, King EJJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with antipyrine. J Clin Pathol 1954; 7:322-330.

25. Malloy HT, Evelyn KA. The determination of bilirubin with the photometric colorimeter. J Biol Chem 1937; 119: 481-490.
26. Wooten IDP (1964) Micro-analysis in medical biochemistry, 4th ed, J and A Churchill Ltd, London, p. 138-140.
27. Lee YS, Cho IJ, Kim JW, Lee MK, Ku SK, Choi JS, Lee HJ. Hepatoprotective effects of blue honeysuckle on CCl4-induced acute liver damaged mice. Food Science and Nutrition 2019; 7:322–338.
28. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351-358.
29. Kakkar P, Das B, Viswanath PN. Modified spectrophotometer assay of SOD. Ind J BiochemBiophys 1984; 95: 51–58.
30. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972; 47: 389-394.
31. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588-590.
32. Habig WH, Pabst MJ, Jakoby WB. Glutathione S transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-7139.
33. Horn HD, Burns FH. Assay of glutathione reductase activity. In: Bergmeyer HV (Ed), Methods of enzymatic analysis. Academic press, New York, USA; 1978. p. 142-146.
34. Ellman GL. Tissue sulfhydryl groups. Arch BiochemBiophys 1959; 82:70–77.

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RESEARCH ARTICLE

Identification of Plant Growth Promoting Candidate Genes (*trpC* and *ipdC*) in *Streptomyces griseoviridis* and *Enterobacter asburiae* whole Genome and evaluation of Plant Growth Potential using *Trigonella foenum-graecum* (Fenugreek)

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ABSTRACT

As plants are immobile, they exhibit various mechanisms to overcome harsh environmental conditions such as predator attack, parasite, pathogen infection and heavy metal accumulation in the soil, which are influenced by association between plants-microbes. In addition, beneficial organisms such as endophytes (bacteria and fungi), are ubiquitously present in all the plants. Therefore, the mechanisms between plant and microbes are imperative to mitigate the harsh environmental conditions that influence plant growth. This study focused to investigate the key role played by soil microbe which in turn helpful to overcome the adverse condition which influence the plant growth. For this, *Streptomyces griseoviridis* (GCA_005222485.1) and *Enterobacter asburiae* (GCA_000799205.1) genomes were selected and analysed using BLAST Ring Image Generator (BRIG) *in-silico* tool. The whole genome analysis revealed the presence of candidate genes *trpC* (Tryptophan biosynthesis protein) and *ipdC* (indole – 3- pyruvate decarboxylase gene) responsible for plant growth promotion and infection. As reported earlier, the *trpC* and *ipdC* genes code for the key enzymes such as indole-3-glycerol synthetase and indole-3-pyruvate decarboxylase (IPDC) involved in the production of indole-3-acetic acid (IAA) which is an important plant growth hormone essential for shooting and rooting of plants even plants in adverse condition. The whole genome analysis results of this study were verified through preliminary experiments using *Trigonella foenum-graecum* (Fenugreek), since, fenugreek being one of the oldest model medicinal plants known for its medicinal and nutritional value. Five different soil samples from different places such as garden, agricultural field, pond, soil dumped with plastic waste and heavy metal contaminated soil were taken. Simultaneously, consortia of different microbes such as *Enterococcus sp., Streptococcus sp., Bacillus*

sp. and *Pseudomonas sp*. were made. Fenugreek seeds was surface sterilized and was made to grown along with the soils collected from different regions in two different conditions; such as the seeds were exposed to the soil samples with and without the microbial consortium. The growth of *Trigonella foenum-graecum* was observed until two leaf stage. The seeds showed short shoot without microbes, whereas with consortia showed linear and upright shoot. These results are evident that treatment with bacterial consortia allowed the plant to tolerate adverse conditions effectively and assisted for better growth. Therefore, the bacterial consortia mentioned in this study could be used as a bio-inoculant for enhancing the growth of *Trigonella foenum-graecum* in soil high levels of plastic wastes and high heavy metals. Further field work such as application of consortia in soil with much pollutants is necessary to confirm the results obtained in this study.

Keywords: plant-microbe interaction, bio-inoculant, abiotic stress tolerance, whole genome, BRIG.

INTRODUCTION

Plants and microbes have been interacting with each other since the microbes helped plants to colonize land more than 450 million years ago. Multicellular host and its associated microbiota as a functional entity is called as "Holobiont" in which evolutionary selection likely occurs between the host and its associated microbiota (Hassani *et al*., 2018). Wide range of beneficial effects of microbiota members on plant health has been reported in several studies (Turner *et al.,* 2013, Mendes *et al.,* 2011). Microbiota also plays an important role in priming the immune system of the plant including disease suppression (Van der Ent *et al.,* 2009), induction of systemic resistance (Zamioudis *et al.,* 2015), increased tolerance to abiotic stresses (Rolli *et al.,* 2015) and adaptation to environmental variations (Haney *et al.,* 2015). Bacterial community establishment is not random but the factors that determine the establishment of bacterial communities in the plant includes the soil type, plant developmental stage and the season of plant growth (Bulgarelli *et al.,* 2012).

Plants on being continuously challenged by various biotic and abiotic stress factors during their life cycle has adapted various mechanism in order to thrive which includes interaction with microbes. The plant microbe interaction can be mutually beneficial outcome but can also result in the microbe trying to invade the plant to complete its life cycle (or) even the opposite, it has recently been reported that plant eat microbes in some cases, plants engulf and digest non-symbiotic and non-pathogenic microbes like *yeast* and *E. coli* and use them as a source for nutrition. Engulfing takes place by the generation of an extracellular cell wall like structure for engulfing the microbes (Paungfoo – Lonhienne *et al.,* 2010).

Important examples of positive plant - microbe interaction associated to plant growth promotions include non-pathogenic *Pseudomonas sp., Bacillus sp*. and *Azotobacter sp.* (Berg *et al*., 2014 and Lugternberg, 2015). *Pseudomonas* that are associated with plant lives as saprophytes on plant surfaces and inside plant tissues. They promote plant growth by suppressing pathogenic micro-organisms, synthesizing growth-stimulating plant hormones (*trpc* and *ipdc***)** and promoting plant - disease resistance (Zhao 2011 and Malhotra 2007, Abramovitch Robert *et al*., 2013). *Bacillus* promote growth by producing phyto hormones and siderophores solubilize phosphate (Tiwari *et al*., 2019, Kang *et al*., 2019). In order to study the pathogenicity potential of pathogens, sequencing and *in-silico* studies are highly useful. Especially, the genomic information of plant pathogens provides the virulence mechanism thereby researcher can plan the perfect disease control strategy. Raskin *et al.,* 2006 rightly reported that genomic information has provided the insight into pathogen and the potential virulence mechanism. Till date, 126 plant pathogenic bacterial species have been sequenced (Jin and Nian, 2019). To explore the plant microbe interaction, comparative genomic analysis between two different strains *S. griseoviridis* (GCA_005222485.1-non-virulent strain) and *E. asburiae* (GCA_000799205.1-virulent strain) was used. To confirm the *in-silico* results, axenic and microbial consortia have

been formulated to examine how the consortia is more effective in promoting plant growth in the presence of pollutants (Kang *et al.,* 2019) or adverse condition. The *Trigonella foenum-graecum* (Fenugreek) model plant was subjected to different soil samples enriched with pollutants and the key role played by soil microbes to overcome stress condition was examined.

MATERIALS AND METHODS

Chemicals

All chemicals were reagent grade and purchased from HI media (http://www.himedialabs.com) chemical company unless otherwise stated.

Bacterial strains and cultivation conditions

Enterococcus sp., Streptococcus sp., Bacillus sp. and *Pseudomonas sp.* obtained from the PG and Research Department of Botany, Lady Doak College was used as a 'test strain' in all the microbiological studies mentioned. Cells were cultivated at 37 ◦C in 500 ml Erlenmeyer flasks containing 100 ml Nutrient Broth. Each experiment was performed in triplicates

Reference Genomes used in this study

National Centre for Biotechnology Information (NCBI) (https://ncbi.nlm.nih.gov) provides access to genomic data and integrated view for whole genomes, complete chromosomes. The complete genomes of *S. griseoviridis* (GCA_005222485.1) and *E. asburiae* (GCA_000799205.1) were downloaded from National Center for Biotechnology Information (NCBI) (Acland *et al*., 2013). GenBank databases were used for the retrieval of protein sequences for *S. griseoviridis* (GCA_005222485.1) and *E. asburiae* (GCA_000799205.1) as reported earlier (David *et al.,* 1998).

Sequence analysis of retrieved data

The retrieved DNA sequences were analyzed using BLAST for pairwise sequence comparisons (Altschul *et al.,* 1997). Multiple Sequence Alignment was generated by Clustal Omega program (Thompson *et al.,* 1994).

BLAST Ring Image Generator (BRIG)

BLAST Ring Image Generator (BRIG) is capable of generating circular genome comparison for prokaryote genomes. It compares multiple genomes at one time as a single image. In BLAST Ring Image Generator (BRIG), the input genome sequences were selected as "input data", followed by " circular ring" window where one or more query sequence file were loaded and chosen for each concentric ring. Image constructing configurations such as ring colour, size, identify thresholds and text can also be specified. Finally, the window settings were confirmed and submitted to Blast Ring Image Generator (BRIG) to perform the genome comparison for *S. griseoviridis* (GCA_005222485.1) and *E.asburiae* (GCA_000799205.1) as reported earlier (Nabril *et al*.,2011). BLAST" is a local alignment tool used to align similar genomes or protein based on the information stored in the database (https://www.ncbi.nlm.nih.gov>blast). Whole genome of *S.griseoviridis* (GCA_005222485.1) and *E.asburiae* (GCA_000799205.1) were run on BLAST for analysis.

"BLAST2 Sequence" (https:// www. ncbi. nlm. nih. gov/gorf/bl2html) is a new BLAST based tool used to compare two protein or nucleotide sequences (Tatiana *et al.,* 1999). The ***trpC*** and ***ipdC*** genes of *S.griseoviridis* (GCA_005222485.1) and *E.asburiae* (GCA_000799205.1) were run on BLAST 2 sequence tool for alignment. The output of BLAST2 sequences consists of a set of the traditional pairwise alignments generated and supplemented with a dot plot. These dot plots are used to highlight deletion, duplications between two sequences (Wheeler and Bhagwat, 2007; https://www.ncbi.nlm. nih.gov/books/NBK1734/)

Clustal Omega

ClustalOmega (https://www.ebi.ac.uk/Tools/msa/clustalo/) (https://www.genome.jp>tools-bin/clustalw) is a Multiple Sequence Alignment (MSA) program which provide phylogenetic tree in computationally efficient and accurate manner. The *trpC* and *ipdC* gene sequences of *Streptomyces griseoviridis* (GCA_005222485.1) and *Enterobacter* subjected to Clustal Omega to confirm the alignment. MSA helps in the phylogenetic analysis of *S. griseoviridis* (GCA_005222485.1) and *E. asburiae* (GCA_000799205.1) as reported earlier (Fabin *et al.,* 2018).

Sample collection

5 gm of soil samples were collected from five different places such as garden, agriculture field, soil dumped with plastic waste and soil contaminated with heavy metal, pond sample in and around Madurai Dt. in a sterile container. One gram of soil sample was dispensed in 1ml of water and mixed vigorously and the supernatant was taken for further work.

Consortium Preparation

Microbial cultures of *Enterococcus sp., Streptococcus sp., Bacillus sp.* and *Pseudomonas sp.* was obtained from the Department of Botany, Lady Doak College Madurai and was revived using nutrient medium. From the overnight culture, 100 µl of each strain (equal proportions) were added to the sterile centrifuge tube containing nutrient broth (Ghazali et al, 2004); the Consortia of microbes made was stored at 4°C.

Surface sterilization of *Trigonella foenum-graecum* (Fenugreek seeds)

Trigonella foenum-graecum (Fenugreek seeds) were soaked in distilled water for 12 hours. The soaked seeds were washed using distilled water and the seeds were exposed to hydrogen peroxide for 1 minute for surface sterilization. The seeds were again rinsed using distilled water and prepared for planting.

Paper Towel method

The collected soil samples were diluted using distilled water in a Petri dish. Tissue paper was taken and on one half of the paper 4 to 5 seeds were placed and the other half was folded over the seeds. The tissue paper was carefully placed on the Petri dish containing the diluted soil samples in such a way that the tissue paper remains moisturized (Figure 1).

Preliminary study on plant and microbe interaction using heavy metal contaminated soil samples

The collected soil samples were diluted using distilled water and 100 micro litres of the consortia was added to the diluted soil samples in a Petri dish. The surface sterilized seeds were taken and placed on one half of the tissue paper and the other half was folded and placed on the Petri dish containing the soil sample (sterilized) and the soil with consortium. The tissue was positioned in such a way that they remain moisturized (Figure 2). The plant growth was examined to demonstrate the key role of consortia in the polluted environment.

RESULT AND DISCUSSION

***In silico* analysis**

BLAST RING IMAGE GENERATOR (BRIG)

BLAST RING IMAGE GENERATOR (BRIG) is a tool used for simple prokaryote genome comparison. Visualisation of genome comparison is used to determine genotypic differences between closely related prokaryotes (Nabril *et al.,* 2011). BLAST RING IMAGE GENERATOR (BRIG) can generate images that show multiple prokaryote genome comparison without any arbitrary limit on the genome compared. Whole genome sequence of *S. griseoviridis* and *E. asburiae* was run in the BRIG tool. Since the genome's are not related to each other no colour rings were generated (Figure 3).

Clustal Omega

Clustal Omega is a multiple sequence alignment tool which is accurate and allows alignment of any size to be produced. Clustal Omega has a number of features for adding sequences to existing elements of array using existing elements to help align new sequences. Based on the *insilico* works done, *trpC* gene and *ipdC* gene found in *S.griseoviridis* and *E. asburiae* which codes for indole-3-glycerol synthetase and indole-3-pyruvate decarboxylase respectively is present in other species of the genus as well. Indole-3-glycerol synthetase and indole-3-pyruvate decarboxylase are key components involved in the synthesis of indole-3-acetic acid (IAA). IAA is a plant growth hormone which plays an important role in rooting during the early stages of plant development (Mariita and Sello, 2018)

Preliminary study on plant and microbe interaction

Plants are very much affected due to various anthropogenic activities which creates a very stressed environment for them to grow. Thus, plants along with the plant growth promoting rhizobacteria such as *Streptomyces sp., Enterococcus sp., Streptococcus sp., Bacillus sp.* and *Pseudomonas sp.* forms a beneficial interaction and overcome the stressful environment. The results obtained from *insilico* analysis demonstrated the presence of *trpC* and *ipdC* genes in *S. griseoviridis* and *E. asburiae* thus helpful to promote plant growth even in the stressful condition. A simple experiment designed to prove the role of microbes in tackling the stressful environment, wherein *Trigonella foenum-graecum* (Fenugreek) seeds were exposed to Consortium containing *Streptomyces sp., Enterococcus sp., Streptococcus sp., Bacillus sp.* and *Pseudomonas sp*. The seeds were placed in a control soil (sterilized), soil enriched with pollutants and heavy metal and each experimental set up was mixed with a consortium (Geetha Rajendran *et al,* 2012). The results clearly showed that seeds grown with consortium indicated the increase in shoot length without any coiling at 2 leaf stage. While the seeds that was not exposed to the Consortium was observed to have coiled and short shoot length (Figure 4).

CONCLUSION

The results of this study clearly showed that the key players i.e., microbes which are very much helpful to boost the plant growth. Therefore, the bacterial consortia containing *Enterococcus sp., Streptococcus sp., Bacillus sp.* and *Pseudomonas sp.* could be used as a bio-inoculant for enhancing the growth of *Trigonella foenum-graecum* in soil containing pollutants and chemicals from outlets of industries. Still, further field work is necessary to confirm the results obtained.

REFERENCES

1. Altschul, Stephen F., et al. "Basic local alignment search tool." *Journal of molecular biology* 215.3 (1990): 403-410.
2. Berg, Gabriele, et al. "Unraveling the plant microbiome: looking back and future perspectives." *Frontiers in microbiology* 5 (2014): 148.
3. Bulgarelli, Davide, et al. "Structure and functions of the bacterial microbiota of plants." *Annual review of plant biology* 64 (2013): 807-838.
4. David, Baliah V., Govindan Chandrasehar, and Pamila N. Selvam. "Pseudomonas fluorescens: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops." *Crop improvement through microbial biotechnology*. Elsevier, 2018. 221-243.
5. Haney, Cara H., et al. "Associations with rhizosphere bacteria can confer an adaptive advantage to plants." *Nature plants* 1.6 (2015): 1-9.
6. Hassani, M. Amine, Paloma Durán, and Stéphane Hacquard. "Microbial interactions within the plant holobiont." *Microbiome* 6.1 (2018): 1-17.
7. JinXu and Nian Wang (2019). Where are we going with genomics in plant pathogenic bacteria? Genomics, Vol.111, Issue 4 P-729-736

8. Kang, Dingrong, et al. "Construction of simplified microbial consortia to degrade recalcitrant materials based on enrichment and dilution-to-extinction cultures." *Frontiers in microbiology* 10 (2020): 3010.
9. Lugtenberg, Ben. "Introduction to plant-microbe interactions." *Principles of Plant-Microbe Interactions*. Springer, Cham, 2015. 1-2.
10. Lugtenberg, Ben. "Life of microbes in the rhizosphere." *Principles of plant-microbe interactions*. Springer, Cham, 2015. 7-15.
11. Mendes, Rodrigo, et al. "Deciphering the rhizosphere microbiome for disease-suppressive bacteria." *Science* 332.6033 (2011): 1097-1100.
12. Paungfoo-Lonhienne, Chanyarat, et al. "Turning the table: plants consume microbes as a source of nutrients." *PLOS one* 5.7 (2010): e11915.
13. Rajendran, Geetha, Maheshwari H. Patel, and Sanket J. Joshi. "Isolation and characterization of nodule-associated Exiguobacterium sp. from the root nodules of fenugreek (Trigonella foenum-graecum) and their possible role in plant growth promotion." *International journal of microbiology* 2012 (2012).
14. Raskin, David M., et al. "Bacterial genomics and pathogen evolution." *Cell* 124.4 (2006): 703-714.
15. Rolli, Eleonora, et al. "Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait." *Environmental microbiology* 17.2 (2015): 316-331.
16. Sievers, Fabian, et al. "Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega." *Molecular systems biology* 7.1 (2011): 539.
17. Tatusova, Tatiana A., and Thomas L. Madden. "BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences." *FEMS microbiology letters* 174.2 (1999): 247-250.
18. Thompson, Julie D., Desmond G. Higgins, and Toby J. Gibson. "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice." *Nucleic acids research* 22.22 (1994): 4673-4680.
19. Tiwari, Shalini, Vivek Prasad, and Charu Lata. "Bacillus: plant growth promoting bacteria for sustainable agriculture and environment." *New and future developments in microbial biotechnology and bioengineering*. Elsevier, 2019. 43-55.
20. Turner, Thomas R., Euan K. James, and Philip S. Poole. "The plant microbiome." *Genome biology* 14.6 (2013): 1-10.
21. Van der Ent, Sjoerd, et al. "Priming of plant innate immunity by rhizobacteria and β-aminobutyric acid: differences and similarities in regulation." *New Phytologist* 183.2 (2009): 419-431.
22. Wheeler, David, and Medha Bhagwat. "BLAST quickstart." *Comparative genomics*. Humana Press, 2007. 149-175.
23. Xie, Yunchang, et al. "Identification and utilization of two important transporters: SgvT1 and SgvT2, for griseoviridin and viridogrisein biosynthesis in *Streptomyces griseoviridis*." *Microbial cell factories* 16.1 (2017): 1-10.
24. Ye, Jianxiong, et al. "Evaluation of a biocontrol preparation consisting of Enterobacter asburiae JX1 and a lytic bacteriophage cocktail to suppress the growth of Salmonella Javiana associated with tomatoes." *Journal of food protection* 72.11 (2009): 2284-2292.
25. Zamioudis, Christos, et al. "Rhizobacterial volatiles and photosynthesis-related signals coordinate MYB 72 expression in Arabidopsis roots during onset of induced systemic resistance and iron-deficiency responses." *The Plant Journal* 84.2 (2015): 309-322.
26. Zhao, Yunde. "Auxin biosynthesis: a simple two-step pathway converts tryptophan to indole-3-acetic acid in plants." *Molecular plant* 5.2 (2012): 334-338.
27. Malhotra, Mandira, and Sheela Srivastava. "An ipdC gene knock-out of Azospirillum brasilense strain SM and its implications on indole-3-acetic acid biosynthesis and plant growth promotion." *Antonie Van Leeuwenhoek* 93.4 (2008): 425-433.

Figure 1: The 12-hr soaked *Trigonella foenum-graecum* seeds on tissue paper.

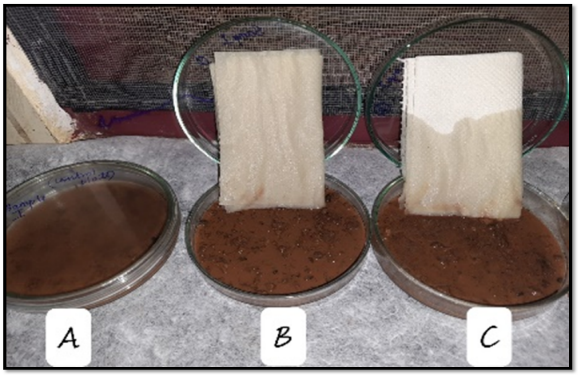

Figure 2: Experimental set up showing the heavy metal contaminated soil treated with consortium A- Control (sterilized); B- Soil Sample without consortium; C- Soil sample with consortium.

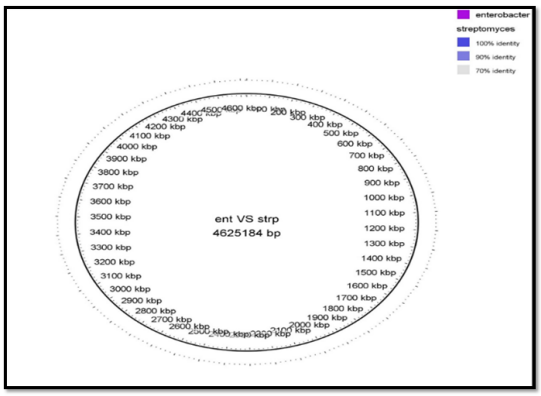

Figure 3: BRIG results of *S.griseoviridis* and *E. asburiae*

Figure 4: Samples 1A, 2A, 3A, 4A, 5A are treated with consortium; Samples 1B, 2B, 3B, 4B, 5B not exposed to consortium

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RESEARCH ARTICLE

Eco- Friendly Polymeric Films from Banana Peels

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ABSTRACT

Bio degradable polymeric film is made out of banana peels taking starch along with glycerol as a plasticizer and turmeric as an antioxidant so as to prevent any antimicrobial activity by blending all together. The mechanical properties such as tensile strength, young's modulus, break point elongation of the dried film was analyzed by tensile tester (Colorcon). Biodegradability of the prepared film was also tested by soil burial method and the morphology of the degradable film was examined by SEM. Also the porosity of the film was measured by water dipping method. The developed film is biodegradable and a suitable alternative for packaging .Also will somewhat help in reducing the pollution caused by plastics.

Keywords: Biodegradable, Banana Peel, Tensile Strength, porosity, packaging

INTRODUCTION

In modern civilization packaging materials like polyethylene carry bags and pouches, PET bottles have gained high importance for their easy processing methods and low cost. But they are regarded as solid waste after use and their improper disposal can cause serious negative impact on marine and terrestrial ecosystem. According to the report of Central Pollution Control Board (CPCB), the total plastic waste generation in India is around 3.3 million metric tonnes per year and contribution of packaging materials is significant in this regard. Due to their non biodegradability they can remain as such for long years and enters into marine as well as terrestrial ecosystem by wind or human activities. Due to heavy demand it is also not possible to stop the manufacturing of these materials and the municipality of different metros across the country has playing a vital role in managing this waste. That's why now-days a number of researches are going on in developing biodegradable packaging materials out of agricultural, vegetable and fruit wastes. In the literature it was reported that banana and orange peels can be suitably converted into biodegradable plastics in presence of cellulose or starch [1]. Antimicrobial properties of the

biodegradable films for food packaging [2] also a major area of focus. Curcumin has been used as an antibacterial agent in many researches and subsequently non toxic [3][4][5]. In this research films were developed out of banana and orange peels by blending with a developer like starch along with glycerol and turmeric using as plasticizer and antimicrobial agent respectively. The properties like biodegradability, tensile strength, break elongation of the film were also analyzed.

EXPERIMENTAL

Materials

Banana peels were collected from domestic waste. Cleaned and washed with water prior to use. Glycerol, Ethanol and acetic acid are reagent grade chemicals and were used as such.

Preparation of film

The banana peels were cut into small pieces with a knife. Then they were boiled in ethanol along with turmeric powder in an reflux condenser for 2 hrs, filtered and dried in an over at 60 ^{0}C.After that made into powder by the help of a blender. To 2.5 g of banana peel powder 4mL of glycerol [6] and 4 mL of acetic acid were added. The mixture was stirred with an electrical stirrer for 45 mins in order to get a uniform paste. The pastes so obtained is spread on a Petridis and dried in over at 60 ^{0}C for 24 hrs.

CHARACTERIZATIONS

Measurement of Hardness

The hardness of the film was measured by Rockwell hardness Tester A300(HR320S), Mitutoya, Brazil at temperature(23±5)^{0}C and relative humidity (50±*10*)% with specimen size 30mm x 30mm x 1mm in HRB Hardness scale. The range of force was 98.07N with minor load 60Kgf and the type of Indenter used was Ball 1/16

Measurement of Mechanical Properties

Mechanical properties of the of the film were determined by tensile tester (Colorcon) specimen size was 4 x 3 cm ^{2} in area and 0.08 ± 0.015mm in thickness and the test speed of the machine was 5mm per minute. Experiments were performed at 22°C and 30-40% RH. Load and displacement values were recorded. Typical test parameters like maximum stress, modulus of elasticity, work done, extension at break and strain at maximum load were automatically computed by software

Porosity

The porosity of the film is determined by dipping in water in a Petridis for 24 hrs and weighing both dry and wet film. Then the films were again weighted by digital weight balance machine and the porosity was determined by the given formula ,

Porosity = W-w /qAl

Where W and w are the weights of the wet and dry films respectively.
q = density of water = 1g cm^{-3}
A = Area of the film
l = thickness of the film

Biodegradability test

The biodegradability of the films were tested by burring the films under soil in ½ feet distance from the ground surface for 10 to 30 days and the films were taken out , cleaned properly with dry cotton cloth, weighed and kept for image analysis by Scanning Electron Microscopy.

SEM

The surface structure of the films was viewed through Scanning Electron Microscope (JEOL 6510 LV, Japan).The membranes were rubbed with tissue paper and then dipped in liquid nitrogen for freezing before analysis.

RESULT AND DISCUSSION

Mechanical Properties Analysis

The mechanical properties like hardness, tensile strength, break point elongation as well as young's modulus of elasticity has been calculated and the data were recorded in table-1. It was observed that all the properties improved with increase in glycerol concentration as shown in figure-2.The weight of the film also increased with addition of glycerol to the reaction mixture as evidenced from figure-2.

Porosity Analysis

From the porosity measurement it was confirmed that there is a decrease in porosity of the film with increase in glycerol content as mentioned in table-2 which may be attributed to the fact glycerol binds firmly to the babana peel surface forming a good and compact structure.

Biodegradability Analysis

The biodegradability of the prepared film was tested by soil burial method and the data has been tabulated in the table-3. It has been found that the percentage of decomposition increased from 10 to 30 days simultaneously .Also the biodegradability increased with increase in conc. of glycerol as shown in the figure-4.

SEM Analysis

The SEM analysis of the film also confirm that the it's degradability percentage in increasing with time as shown in Figure-4. From Image 4(b) is it clear that the degradability of the film has been started though the percentage is less but from image 5(c) and (d) it is confirmed that the film has been degraded a lot upto 30% as mentioned in the table-3

CONCLUSION

Ecofriendly polymeric film was successfully developed from banana peels that are easily available. The method of development or preparation is also cost effective and can easily prepared without need of any sophisticated instrument in the laboratory. The tensile strength and young's modulus value says that the film keeps a load bearing capacity and can withstand small loads. From the porosity result it was confirmed that there are less pore formation with increase in glycerol concentration in the structure. Moreover the biodegradability test was impressive as percentage of decomposition under soil has been increased with time as well as glycerol concentration, confirming the ecofriendly used so as to have a positive impact on soil and water pollution. Hence the developed biodegradable film may be suitable for packaging applications and environmental friendly in near future.

REFERENCES

1. Maria Assunta Acquavia, Raffaella Pascale, Giuseppe Martelli, Marcella Bondoni and Giuliana Bianco1, "Natural Polymeric Materials: A Solution to Plastic Pollution from the Agro-Food Sector", Polymers (Basel), 13(1), 158, (2021)
2. P. Astuti, A. A. Erprihana, "Antimicrobial Edible Film from Banana Peels as Food Packaging", American Journal of Oil and Chemical Technologies, 2, 65-70,(2014)
3. Sin-Yeang Teow , Kitson Liew, Syed A. Ali, Alan Soo-Beng Khoo and Suat-Cheng Peh, "Antibacterial Action of Curcumin against Staphylococcus aureus: A Brief Review", Journal of Tropical Medicine,.2016,1-10,(2016).
4. Gunes, H., Gulen, D., Mutlu, R., Gumus, A., Tas, T., & Topkaya, A. EAntibacterial effects of curcumin. Toxicology and Industrial Health, 32(2), 246–250,(2013).
5. Ilya Shlarab, Samir Drobya, Ruplal Choudharyc and Victor Rodov, "The mode of antimicrobial action of curcumin depends on the delivery system: monolithic nanoparticles vs. supramolecular inclusion complex", RSC Adv., 7, 42559-42569,(2017)
6. M.R.GAONKAR, PRASHANT PALASKAR, RISHIKESH NAVANDAR, "PRODUCTION OF BIOPLASTIC FROM BANANA PEELS", International Journal of Advances in Science Engineering and Technology,6(1) ,36-38,(2018)

Table-1: Mechanical Properties of the film

Amount of glycerol (mL)	Wt. of the film (g)	Hardness(HRB)	Tensile strength (N/mm^2)	Break elongation (%)	Young's modulus($N-mm^2$)
18	2.65	32.6	35.32	435.65	765.23
22	2.67	39.7	38.71	542.55	823.18
26	2.70	45.5	42.47	556.38	871.37

Table-2: Porosity measurement of the film

Vol. of Gylcerol (mL)	Porosity
18	0.053
22	0.032
26	0.026

Table-3: Biodegradability test of the film

Amount of Glycerol added (mL)	Wt.of dry film (g)	% of Decomposition		
		10days	20 days	30 days
18	2.65	10.22	19.32	28.94
22	2.67	11.81	21.54	30.32
26	2.70	13.63	22.92	34.18

Figure 1.Film Preparation

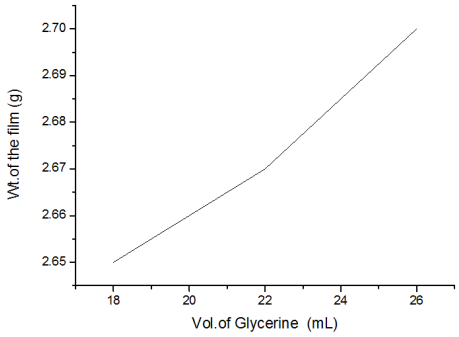

Figure 2. Weight gain against Vol. of Glycerol added

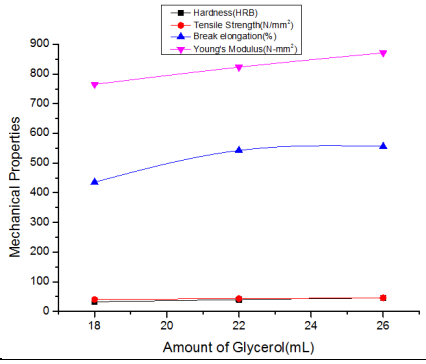

Figure 3. Mechanical Property analysis of the film

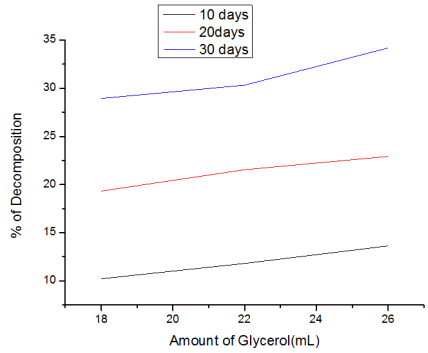

Figure 4. Biodegradability comparison of the film

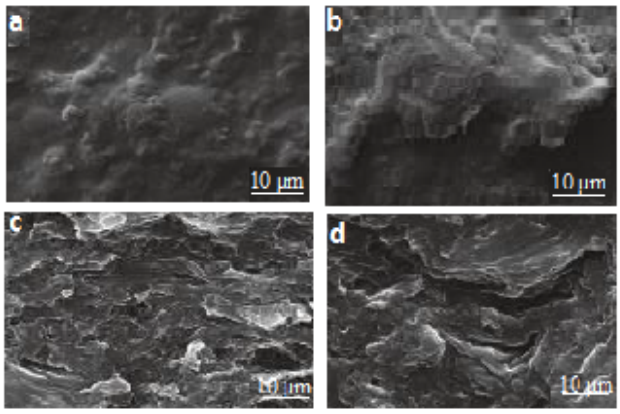

Figure 5. SEM images of the soil buried films after (a)0 (b)10 (c)20 (d)30days

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RESEARCH ARTICLE

Efficacy of Phytol from *Azadirachta indica* A. Juss. against SARS-CoV-2 Virus- An *In silico* Approach

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ABSTRACT

Coronavirus is a new public health crisis threatening the world and it is considered as a major source of catastrophe in the 21st century. To overcome this disaster, there is an urge to develop preventive and curative measures with specific drugs. In this regard, plant-based medicines are in high demand due to its reduced side effects and therapeutic properties when compared to existing synthetic drugs. The meliaceous plant *Azadirachta indica* A. Juss. native to India is chosen for this study due to its medicinal properties and potential phytoconstituents. Among the effective compounds present in the plant, an important phytocompound screened from the leaf extracts using organic solvents by GCMS analysis (i.e.) phytol (diterpenoid) is found to possess anti-microbial, anti-inflammatory, antioxidant and anti-viral properties. It is an excellent immuno-stimulant and activates both innate and acquired immunity. The current study is focused to study the activity of phytol as a ligand against the SARS-CoV-2 targets and compared it with the drug preferred during the covid period (hydroxychloroquine). Phytol and hydroxychloroquine were retrieved from Pubchem database and SARS-CoV-2 targets i.e. Spike glycoprotein, Main protease and RNA dependent RNA polymerase responsible for viral entry and replication were retrieved from Protein Data Bank. Physicochemical analysis, secondary structure prediction and active site of target protein were also analyzed before docking. Both ligand and target docked using PatchDock software had revealed that the phytol potency is higher than the hydroxychloroquine based on docking score, atomic contact energy and best docking fit. Different parts

of *A. indica* have been widely practiced in Ayurvedic and Siddha system of medicine for various ailments since ancient times. From this *in silico* results, it is evident that phytol as one of the constituent in decoctions and formulations will enhance immunity by preventing viral entry and replication which will be useful in the management of infection caused by the SARS-CoV-2 virus.

Keywords: *Azadirachta indica*, Phytol, Hydroxychloroquine, SARS-CoV-2, PatchDock.

INTRODUCTION

Natural compounds derived from plants possess a rich source of novel drugs that cure the ailments of mankind as an important constituent in "Traditional System of Medicine, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates and lead compounds in synthetic drugs". World Health Organization (WHO) has pointed out that plant-based medicines are in high demand, and they are widely used as a source of primary health care by 80% of the global population [1]. Approximately a quarter of drugs approved by the Food and Drug Administration (FDA) are plant-based, revealing the significance of phytocompounds in medicine. Medicinal plants are considered as a good source of anti-viral compounds due to its potential phytoconstituents with proven therapeutic properties against several viruses including SARS-CoV-2 virus [2]. However, overexploitation of medicinal plants for drug development may leads to extinction and inclusion of such plants in Red data book [1]. In such a case, meliaceous plant *Azadirachta indica* A. Juss. which is widely distributed in India and found abundantly in nature is selected as a potential plant for inhibiting the spread of Covid 19.

Azadirachta indica A. Juss. is a multifaceted tree that belongs to the family Meliaceae is considered as "one of the most promising tree of the 21st century" for its medicinal and pesticidal properties by World Health Organization [3]. Neem leaf is a depository of active ingredients which contains nimbin, nimbanene, nimbolide, nimbandiol, n-hexacosanol, ascorbic acid, nimbiol etc. [4]. Almost all parts of *Azadirachta indica* has been extensively utilized in Ayurveda, Homeopathy and Unani system of medicine to control several diseases like leprosy, constipation and skin infections. It also possesses pharmacological activities such as anti-viral, antibacterial, antifungal, antipyretic, anti-inflammatory, antioxidant, antiulcer, antiplasmodial, antidiabetic and antiseptic properties [5, 6]. Studies have reported that neem extracts has the potential to inhibit the replication of several viruses such as bovine herpes virus type-1, Human Immunodeficiency Virus (HIV), poliovirus type 1, duck plague virus, dengue virus type-2, newcastle disease virus, infectious bursal disease virus, avian influenza virus and group B coxsackie virus [7].

Covid 19 is caused by Severe Acute Respiratory Syndrome Corona virus (SARS-CoV-2). It is considered as a major global health issue that belongs to the genus Betacoronavirus, a positive-sense single-stranded RNA known to infect humans, bats and other mammals [8]. World Health Organization declared Covid a "Global pandemic in March 2020" due to an increased mortality rate [9]. In the early days of 2021, over 100 million cases and more than 2 million deaths were reported globally [10]. Novel SARS-CoV- 2 virus consists of several structural proteins such as spike protein (S), an envelope protein (E), membrane protein (M) and nucleocapsid protein (N) embedded with an RNA genome to form the viral particle and non-structural proteins such as proteases and RNA dependent RNA polymerase (RdRp) enzymes which are essential for receptor binding, cellular entry, viral replication and cell to cell spread [11]. The Spike glycoprotein plays a critical role in the process of SARS-CoV-2 invading host cells. The Main protease and RdRp are responsible for replication of SARS-CoV-2 virus [12]. As a result, the Spike glycoprotein, Main protease and RdRp are promising anti-SARS-CoV-2 drug targets, providing ideas for the development of antibodies, drugs and vaccines.

Molecular docking is a powerful technique widely used for drug development and structure-based drug discovery from potent phytocompounds which reduces the cost, time and human intervention [13]. Due to the existing antiviral properties and effectiveness of *Azadirachta indica*, researchers have focused on different parts of neem tree

for discovering drugs against SARS-CoV-2 virus. Thus, the current studies objective is to check the potency of the phytocompound phytol screened from neem leaves as a ligand against SARS-CoV-2 targets (Spike glycoprotein, Main Protease, RdRp) and to compare it with the existing drug hydroxychloroquine which is being used for the control of SARS-CoV-2 virus.

MATERIALS AND METHODS

Collection of plant material

Fresh and mature leaves from disease free *Azadirachta indica* tree species were collected from the Campus of Ethiraj College for Women, Chennai, Tamil Nadu (Latitude N 13° 3´ 49.9716´´; Longitude E 80° 15´ 29.5776´´).

Preparation of crude leaf powder

The leaves collected from *Azadirachta indica* tree species were thoroughly washed with distilled water to remove dust particles. Further leaves were dried in shade and powdered using a blender. The fine powder was transferred to an airtight container for further extraction [14].

Preparation of extract

The crude extract (1:10 w/v) were prepared using different solvents such as petroleum ether, ethyl acetate, acetone, methanol and distilled water by cold percolation method [15]. After 24 hours of soaking, Whatmann No: 1 filter paper was used for filtration. The extract obtained was used for phytochemical analysis.

Qualitative phytochemical screening

The different leaf extracts of *Azadirachta indica* were checked for the presence of phytoconstituents such as carbohydrates, alkaloids, glycosides, phenolic compounds, flavonoids, tannins, terpenoids and saponins using standard procedures [16, 17].

Quantitative estimation of terpenoids

The methanol leaf extract of *A.indica* showed strong presence of terpenoids which was taken for further quantification study using Ferguson's method [18].

GC-MS analysis

The methanol leaf extract (extracted using HPLC graded methanol) was used for GC-MS analysis to find out the presence of biologically active volatile compounds. The sample was analyzed at Sophisticated Instrumentation Facility (SIF), Chemistry Division, School of Advanced Science, VIT University, Vellore- 632 014.

Gas chromatograph

The Clarus 680 GC equipped with a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df).Helium was used as carrier gas to separate the components at a flow rate of 1 ml/min constantly. During the chromatographic run, 260°C was set as injector temperature and 1μl of the extract was injected for analysis. The oven temperature was 60 °C for 2 mins followed by 300 °C at the rate of 10 °C min^{-1}; and 300 °C for 6 mins.

Mass spectrum

The mass detector conditions were transfer line temperature 240 °C; ion source temperature 240 °C; ionization mode electron impact at 70 eV with a scan time 0.2 sec and 0.1 sec, then the fragment from 40 to 600 Da.

Mass spectrometry library search

The spectrum of the components was compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library.

***In silico* studies**

In silico analysis through molecular docking plays a vital role in studying the functional role of selected bioactive phytocompound against SARS-CoV-2 targets. Prior to docking, it is very important to analyze the physicochemical characteristics, secondary structure and prediction of active site of target protein.

Physico chemical characterization

The analysis of physico chemical characteristics of target protein was carried out using ProtParam server. Various parameters such as Molecular weight, Theoretical (pI), Total number of positive and negative charged residues, Extinction coefficient (EC), Instability index (II), Aliphatic index (AI), Grand average of hydropathicity (GRAVY) were computed [19].

Analysis of Secondary structure

The secondary structure of the target protein was determined using Self Optimized Prediction Method with Alignment (SOPMA) to analyze the percentage of alpha helix, beta bridge, extended strand and beta turn present in protein [20].

Active site prediction

Active site in the target protein was determined using "The Computed Atlas of Surface Topography of proteins" (CASTp) server. CASTp provides detailed quantitative characterization of topographic features of proteins. The active site of the amino acids is required for further molecular docking studies [21].

Preparation of ligand and target proteins

From the GCMS analysis of methanolic leaf extract of *A. indica*, a phytcompound which possesses potent antiviral property and lies in good probability range was selected for docking. The three dimensional structure of phytocompound phytol was retrieved from Pubchem database in SDF format and converted into PDB format using Open babel. Similarly, hydroxychloroquine was also retrieved which was used as a positive control (Figure: 1).The three dimensional structure of target proteins such as Spike glycoprotein (PDB ID: 6VXX), Main protease (PDB ID: 6LU7) and RNA dependent RNA polymerase (RdRp) (PDB ID: 6M71) were retrieved from Protein Data Bank (Figure: 2).

Docking Studies

Molecular docking analysis was performed to identify the interaction nature of ligand molecules against the target. The bioactive phytocompound (ligand) was docked against the target proteins using the bioinformatics tool PatchDock [22]. Docking results exhibited the interaction between the target and ligand molecules. Docking studies were carried out to assess the anti-viral property of the phytocompound with the help of binding affinity between ligand and target. Hydroxychloroquine was used as a positive control. From the docking results**,** protein ligand interactions were analyzed using visualization tool Chimera (www.cgl.ucsf.edu/chimera).

RESULTS AND DISCUSSION

Qualitative phytochemical screening

In this study, the phytochemical analysis carried out using different solvents (petroleum ether, ethyl acetate, acetone, methanol and aqueous) has revealed several phytocompounds more prominently in methanol when compared to aqueous and other organic solvents (Table: 1).

All the phytoconstituents such as carbohydrates, alkaloids, glycosides, phenolic compounds, flavonoids, tannins, terpenoids and saponins were present in the methanol leaf extract of *A. indica.* Among the phytoconstituents, the presence of terpenoids were prominent in the methanol leaf extract of *A. indica.* The phytoconstituents are of great demand in enhancing human health. Natural products from plants exhibited a wide range of biological activities in human therapy, veterinary, agriculture, scientific research and other areas due to the presence of secondary metabolites such as alkaloids, terpenoids, phenolic compounds and flavonoids. Alkaloids play a vital metabolic role and controls development in living system and used as medicine in the form of steroidal alkaloids. Terpenoids belongs to natural isoprenoids which possess strong anti-microbial properties making them a compound with promising therapeutic value [23]. Phenolic compounds and Flavonoids are strong antioxidants which prevent oxidative damage of DNA, lipids and proteins responsible for chronic diseases such as cancer and cardiovascular diseases. It also reported to inhibit initiation, promotion and progression of tumors and reduces the risk of hormone related cancers by modulating steroid hormone level in the body [24]. Other phytocompounds such as carbohydrates, glycosides, tannins and saponins make it a valuable plant to use it in medical fields to cure several diseases.

Quantitative estimation of terpenoids

The total terpenoid content of methanolic leaf extract of *Azadirachta indica* was screened for quantification. The results had revealed that the methanol leaf extract of *A. indica* had 88 ± 0.57 mg/g of terpenoids respectively. Terpenoids belong to the largest class of secondary metabolites synthesized from Acetyl-CoA [25]. Terpenoids are considered as one of the most important families of phytocompounds known for its medicinal value. The higher amount of total terpenoid content in plant plays a significant role in curing diseases due to its antibacterial, antifungal, antiviral, anti-inflammatory and antitumour properties [26]. Another important activity of terpenoids are as antioxidants, which supports the function of other antioxidants such as α- tocopherol by employing synergistic effects [27].

GC-MS analysis

GC-MS analysis was carried out in methanol leaf extract of *A.indica* based on the significant amount of the presence of terpenoids. Several peaks were obtained in the GC-MS analysis, indicating the presence of diverse volatile secondary metabolites such as phytol, linolenic acid and palmitic acid (Figure: 3). These bioactive compounds were identified using NIST database on comparison with actual mass spectral obtained. The present study strongly supports the therapeutic properties such as anti-viral, antioxidant, anti-inflammatory, antinociceptive and antiallergic properties of *A. indica* due to the presence of maximum content of terpenoids reported from the analysis. Phytol is one of the identified phytoconstituent selected from the GC-MS analysis, which lies in a good probability range and exhibits potent therapeutic activity for docking studies. Phytol is a valuable diterpenoid regarded as a novel class of phytocompound in pharmaceutical industries which possess therapeutic activity against anxiety and reported to have anti-microbial, antioxidant, antinociceptive and antiallergic properties [28]. Recent studies revealed that phytol is an excellent immuno-stimulant that activates both adaptive and innate immunity [29].

It was evident from the previous studies that neem leaves possess strong antiviral property due to the presence of structurally diverse terpenoids. The crude acidic extract of neem leaves possesses remarkable antiviral activity of 90.38% against herpes simplex virus type 1 compared with acyclovir (54.33%) at a concentration of 20µg/mL [30]. The methanolic extract of neem leaves completely inhibited plaque formation of six serotypes of Coxsackie group B virus (CVB1 - CVB6) at a concentration of 1000µg/mL at 96 hours. It also helps in controlling intracellular replication of virus [31].

***In silico* studies**

Physico chemical characterization

The physical and chemical properties of the target proteins resulted in Table: 2 was analyzed using a web server ProtParam. The extinction coefficient had revealed the amount of light absorbed by a protein at certain wavelength. The target proteins were found to absorb light at 280nm, and hence this wavelength was used for calculation of extinction coefficient. The half-life of the protein is 1.9 hours in *in vitro* mammalian reticulocytes where the time taken by the protein to disappear after its synthesis in a cell. From this results, the instability and aliphatic index has revealed that the target proteins are heat stable in nature [19].

Analysis of Secondary structure

Secondary structure of protein is folded in nature, which was formed due to the backbone atoms interacting with each other. The common secondary structures are alpha helix, beta sheets and coils. The prediction of secondary structure is an essential intermediate step to predict the three dimensional structure of protein and its function. The secondary structure of the protein was determined using SOPMA with default parameters such as window width 17, similarity threshold 8 and number of states 4 were used for the study. The results of secondary structure of target proteins were depicted in Table: 3 [20, 32].

Active site prediction

It is important to determine the presence of amino acids in active site of the target proteins for docking studies. Figure 4 has revealed CASTp results of the amino acids that are in the active site region of target proteins. The amino acid residues present in the binding pocket of 6VXX (THR 747, GLU 748, ASN 751 & PRO 986); 6LU7 (SER 10 & VAL 125) and 6M71 (ASN 496, LYS 545, GLY 590, SER 592, PHE 594, ALA 688, LEU 758, CYS 813, ARG 836, ASP 845 & GLN 932) were reported.

Docking studies

Molecular docking analysis was carried out using PatchDock software which estimates the binding energy between a target protein and the ligand. From each spatial conformation, the lead molecule having a better docking score is further filtered and viewed using Chimera. Different structural poses were obtained out of which the topmost pose, best docking score and atomic contact energy were screened and analyzed. The phytocompound phytol and positive control hydroxychloroquine was docked against targets such as Spike glycoprotein (6VXX), Main protease (6LU7) and RNA dependent RNA polymerase (6M71). The PatchDock results had revealed the docking score and atomic contact energy of phytol which was found to be predominantly higher than the positive control against all the targets(Figure: 5,6). The docking score and the highest negative value of atomic contact energy exhibited that the phytocompound phytol was in good fit with the target in three-dimensional space to inhibit the activity of viral replication. Among the targets, phytol exhibited higher docking score against the target 6VXX which has clearly indicated that it has the ability to prevent the invasion of spike glycoprotein into the host cell membrane.

Similar studies carried out using phytocompounds such as azadirachtin, epoxyazadiradione, gedunin and nimbin of *Azadirachta indica* when docked against three targets of SARS-CoV-2 virus (6VSB - Surface glycoprotein responsible for viral attachment; 6M71 - RNA dependent RNA polymerase responsible for viral replication; 6Y84 - main protease responsible for viral replication), gedunin exhibited highest binding efficacy with the binding energy of -7.78 kcal/mol, -7.61 kcal/mol and -9.40 kcal/mol against 6VSB, 6M71 and 6Y84 targets[33]. Several compounds such as nimocin, nimbolin A, 7-deacetyl-7-benzoylgedunin, 24-methylenecycloartanol and cycloeucalenone derived from *A. indica* were docked against E protein of SARS-CoV-2. Nimbolin A revealed the strongest binding energy -11.2 kcal/mol against E protein [2]. From this, it is evident that a specific ligand shows good binding affinity with the specific target. Similarly, different targets of SARS-CoV-2 are inhibited by different ligands of the same plant.

Considering the dreadful global issue during Covid 19, neem leaves were extensively used by people in various ways due to its traditional knowledge and therapeutic properties. Ministry of AYUSH has also recommended medicinal plants for management of Covid-19. As a primary step, Fumigation and sanitization of natural herbs using *Azadirachta indica* were practiced [34]. Combination of neem leaves, ginger, garlic and lemon boiled for 30 minutes and steam-inhaled which helps in prevention and control of lung infection [35]. Oral intake of minimal number of young neem leaves helps in regulating blood sugar level in the body which is also one of the ideal condition for SARS-CoV-2 infection [36]. The aqueous leaf extract of neem infusion successfully improves antibody titre which blocks the replication of virus [7]. *Insilico* studies confirms the potency of the promising phytocompounds present in the leaves of *Azadirachta indica* for the prevention and management of SARS-CoV-2 virus as indicated in traditional system of medicine.

CONCLUSION

The development of novel anti-viral molecule is a global priority, mainly for those viral diseases without specific treatment. Computer-aided drug discovery (CADD) methods are based on both ligand and target interaction which will pave the way for drug design with low cost and time associated with conventional methods. It is clear that the continuous spread of Covid 19 has infected many lives. In India, Ayurveda and Siddha system being the ancient medical science has established a great pivotal role in disease management. From this *in silico* results, it is evident that the presence of such potential phytocompounds (phytol) in decoctions and formulations must be one of the reason for enhancing the immunity due to its anti-viral and immuno-stimulant properties with no side effects that prevents viral entry as well as replication leading to the management of infection caused by SARS-CoV-2 virus.

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REFERENCES

1. Bijauliya RK, Alok S, Chanchal DK, Sabharwal M, Yadav RD. An updated review of pharmacological studies on *Azadirachta indica* (neem). International Journal of Pharmaceutical Sciences and Research 2018;9(7):2645-2655.
2. Borkotoky S, Banerjee M. A computational prediction of SARS-CoV-2 structural protein inhibitors from *Azadirachta indica* (Neem). Journal of Biomolecular Structure and Dynamics 2020;1-17.
3. Dubey S, Kashyap P. *Azadirachta indica*: A plant with versatile potential. RGUHS Journal of Pharmaceutical Sciences 2014;4(2):39-46.
4. Chauhan M, Mani P, Sharma TK. The antibacterial activities of neem [*Azadirachta indica*] seed Oil, a review. IOSR Journal of Applied Chemistry 2018;11(5):58-63.
5. Haque SE, Sreenivasulu M. *Azadirachta indica* and its antidiabetic potential - a review. International Journal of Pharmacognosy 2016;3(7):288-294.
6. Prashanth GK, Krishnaiah GM. Chemical composition of the leaves of *Azadirachta indica* Linn (Neem). International Journal of Advancement in Engineering and Technology, Management and Applied Science 2014;1(3):21-31.

7. Muderawan IW, Budiawan IM, Giri MKW. The Potential of Ayurvedic Medicinal Plants for Prevention and Therapeutic Treatment of Covid-19: A Review Article. International Journal of Ayurvedic and Herbal Medicine 2021;11(2):3954–3996.
8. Aftab SO, Ghouri MZ, Masood MU, Haider Z, Khan Z, Ahmad A ***et al***. Analysis of SARS-CoV-2 RNA-dependent RNA polymerase as a potential therapeutic drug target using a computational approach. Journal of Translational Medicine 2020;18(1):1-15.
9. Jiang Y, Yin W, Xu HE. RNA-dependent RNA polymerase: Structure, mechanism, and drug discovery for COVID-19. Biochemical and Biophysical Research Communications 2020;1-14.
10. Nesari TM, Bhardwaj A, ShriKrishna R, Ruknuddin, G., Ghildiyal, S., Das A ***et al.*** Neem (*Azadirachta indica* A. Juss) Capsules for Prophylaxis of COVID-19 Infection: A Pilot, Double-Blind, Randomized Controlled Trial. Alternative Therapies in Health and Medicine 2021.
11. Chatterjee SK, Saha S, Munoz MNM. Molecular pathogenesis, immunopathogenesis and novel therapeutic strategy against COVID-19. Frontiers in molecular biosciences 2020;7:196.
12. Wang MY, Zhao R, Gao LJ, Gao XF, Wang DP, Cao JM. SARS-CoV-2: structure, biology, and structure-based therapeutics development. Frontiers in cellular and infection microbiology 2020;10:587269.
13. Mammen M, Mohanan AG, Kumar P. Computational and experimental validation of methotrexate as Staphylococcal-DHFR inhibitor. Current Trends in Biotechnology and Pharmacy 2020;14(4):396-402.
14. Al-Hashemi ZS, Hossain MA. Biological activities of different neem leaf crude extracts used locally in Ayurvedic medicine. Pacific Science Review A: Natural Science and Engineering 2016;18(2):128-31.
15. Eloff JN. Which extractant should be used for the screening and isolation of anti-microbial components from plants?.Journal of Ethnopharmacology 1998;60(1):1-8.
16. Harborne, AJ. Phytochemical methods a guide to modern techniques of plant analysis. 3rd ed. Springer Science & business media; 1998.
17. Raaman, N. Phytochemical techniques. New India Publishing; 2006. p. 19-24.
18. Valli SA, Gowrie SU. A study on the bioactive potential of fresh and dried coconut sprouts of *Cocos nucifera* L. an in vitro and in-silico approach. International Journal of Pharmacy and Pharmaceutical Sciences 2017;9(3):129-142.
19. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the ExPASy server. The proteomics protocols handbook 2005;571-607.
20. Ji YY, Li YQ. The role of secondary structure in protein structure selection. The European Physical Journal E 2010;32(1):103-107.
21. Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: computed atlas of surface topography of proteins. Nucleic acids research 2018;46(W1):W363-W367.
22. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson, HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic acids research 2005;33(suppl 2):W363-W367.
23. Sinaga M, Ganesan K, Nair SKP, Gani SB. Preliminary phytochemical analysis and in vitro antibacterial activity of bark and seeds of Ethiopian neem (*Azadirachta indica* A. Juss). World Journal of Pharmacy and Pharmaceutical Sciences 2016;5(4);1714-1723.
24. Hollman, PCH. Evidence for health benefits of plant phenols: local or systemic effects?. Journal of the Science of Food and Agriculture 2001;81(9):842-852.
25. Taiz L, Zeiger E, Moller IM,Murphy, A. Plant physiology and development. 6th ed.Sinauer Associates Incorporated; 2015. p.A41-A417.
26. Hortelano S. Molecular basis of the anti-inflammatory effects of terpenoids. Inflammation & Allergy-Drug Targets 2009;8(1):28-39.
27. Chathurdevi G, Gowrie SU. A promising source of potential bioactive metabolites from the root extracts of *Casuarina junghuhniana*. International Journal of Current Research 2016;8(12): 43393-43401.

28. Sinan KI, Etienne OK, Stefanucci A, Mollica A, Mahomoodally MF, Jugreet S ***et al***. Chemodiversity and biological activity of essential oils from three species from the Euphorbia genus. Flavour and Fragrance Journal 2021;36(1):148-158.
29. Balasubramanian S, Ganesh D, Surya Narayana VVS. GC-MS analysis of phytocomponents in the methanolic extract of *Azadirachta indica* (Neem). Int J Pharm Bio Sci 2014;5(4):258-262.
30. Amer H, Helmy WA, Taie HA. In vitro antitumor and antiviral activities of seeds and leaves Neem (*Azadirachta indica*) extracts. Int. J. Acad. Res 2010;2(2):47-51.
31. Badam L, Joshi SP, Bedekar SS. 'In vitro'antiviral activity of neem (*Azadirachta indica*. A. Juss) leaf extract against group B coxsackieviruses. The Journal of communicable diseases 1999; 31(2):79-90.
32. Sumitha A, Devi PB, Hari S, Dhanasekaran R. COVID-19 - In Silico Structure Prediction and Molecular Docking Studies with Doxycycline and Quinine. Biomedical and Pharmacology Journal 2020;13(3):185-1194.
33. Kumar A. Molecular docking of natural compounds from tulsi (*Ocimum sanctum*) and neem (*Azadirachta indica*) against SARS-CoV-2 protein targets. BEMS Reports;6(1):11-13.
34. Kumar V, Singh SB, Singh S. COVID-19: Environment concern and impact of Indian medicinal system. Journal of Environmental Chemical Engineering 2020;8(5):104144.
35. Orisakwe OE, Orish CN, Nwanaforo EO. Coronavirus disease (COVID-19) and Africa: acclaimed home remedies. Scientific African 2020;e00620.
36. Srivastava AK, Chaurasia JP, Khan R, Dhand C, Verma S. Role of medicinal plants of traditional use in recuperating devastating COVID-19 situation. Med Aromat Plants (Los Angeles) 2020;9(359):2167-0412.

Table 1: Qualitative Phytochemical Analysis of Leaf Extracts of *Azadirachta indica*

Phytoconstituents	Leaf extracts of *Azadirachta indica*				
	PE	EA	A	M	AQ
1.Carbohydrates	-	-	+	+	-
2.Alkaloids	+	-	+	+	+
3.Glycosides	-	-	-	+	+
4.Phenolic compounds	-	-	+	+	+
5.Flavonoids	+	+	+	+	+
6.Tannins	-	-	-	+	-
7.Triterpenoids	-	-	+	++	+
8.Saponins	-	-	+	+	-

* PE = Petroleum ether; EA = Ethyl acetate; A = Acetone; M = Methanol and AQ = Aqueous

*(+) = Present; (++) = strongly present; (-) = absent.

Table 2: Physico Chemical Characteristics of SARS-CoV-2 Proteins (6VXX, 6LU7 and 6M71)

S. No	Physico chemical parameters	SARS-CoV-2 TARGET PROTEINS		
		6VXX	6LU7	6M71
1.	Molecular weight (Da)	141410.94	33796.64	108031.65
2.	Theoretical pI (Isoelectric point)	6.09	5.95	6.33
3.	Extinction coefficient(M^{-1} cm^{-1})	138825	33640	137670
4.	Instability index	31.26	27.65	28.13
5.	Aliphatic index	83.32	82.12	77.60
6.	Grand average of hydropathicity (GRAVY)	-0.139	-0.019	-0.256
7.	Total no of negatively charged residues	111	26	106
8.	Total no of positively charged residues	99	22	94

Table 3: Analysis of Secondary Structure of SARS-CoV-2 Proteins (6VXX, 6LU7 and 6M71)

S.No	Analysis of Secondary Structure	SARS-CoV-2 TARGET PROTEINS		
		6VXX (%)	6LU7 (%)	6M71 (%)
1.	Alpha helix (Hh)	28.96	29.08	41.30
2.	Extended strand (Ee)	21.31	27.12	19.32
3.	Beta turn (Tt)	4.76	11.44	7.54
4.	Random coil (Cc)	44.96	32.35	31.85

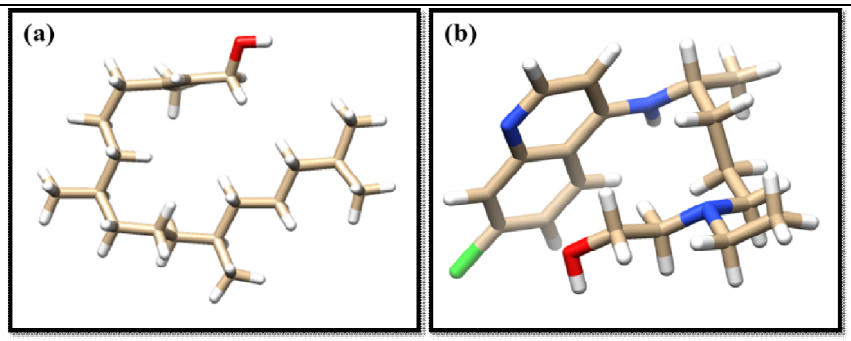

Figure 1: Three Dimensional structure of (a) Phytol(b) Hydroxychloroquine

Figure 2: Three Dimensional structure of target proteins (a) 6VXX (b) 6LU7 (c) 6M71

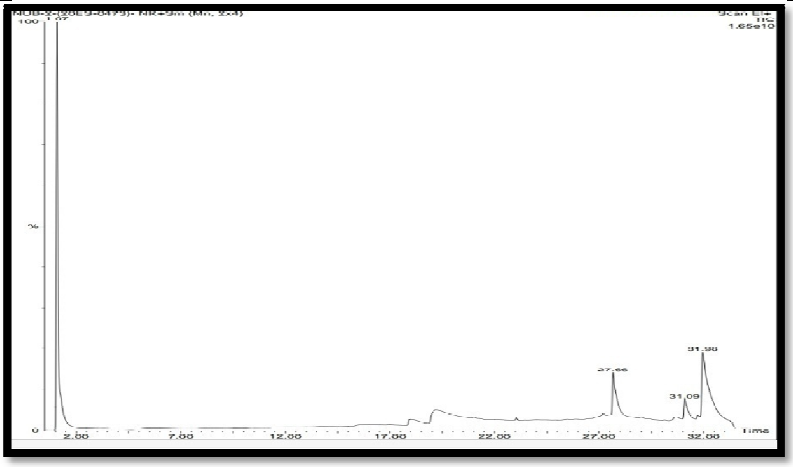

Figure 3: GC-MS spectrum of methanolic leaf extract of *Azadirachta indica*

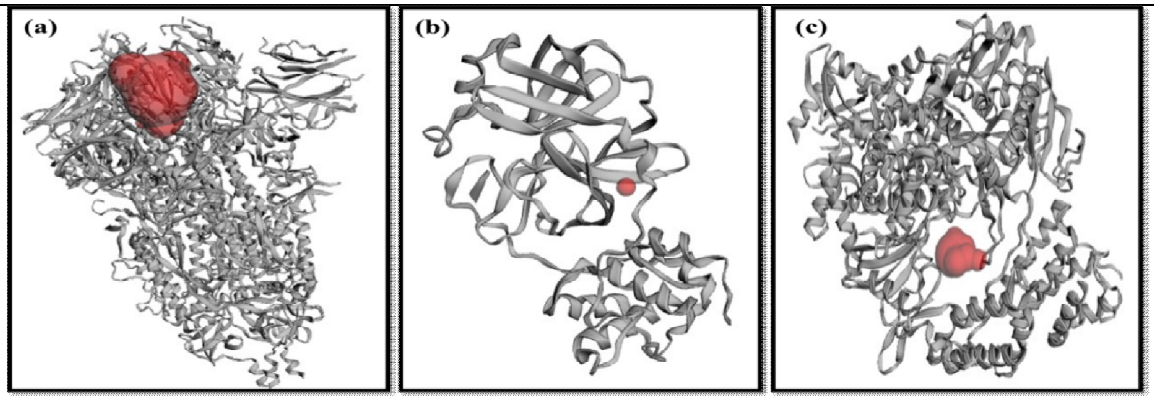

Figure 4: Binding pockets of target proteins (a) 6VXX (b) 6LU7 (c) 6M71

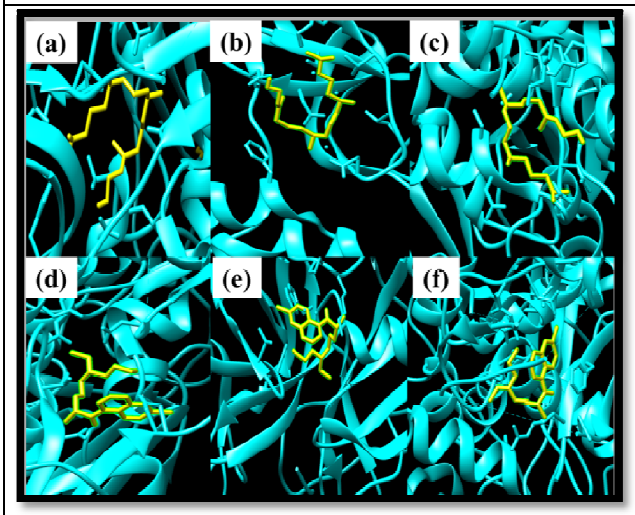

Figure 5: Docking of (a) phytol against 6VXX (b) phytol against 6LU7(c) phytol against 6M71(d) hydroxychloroquine against 6VXX (e) hydroxychloroquine against 6LU7(f) hydroxychloroquine against 6M71

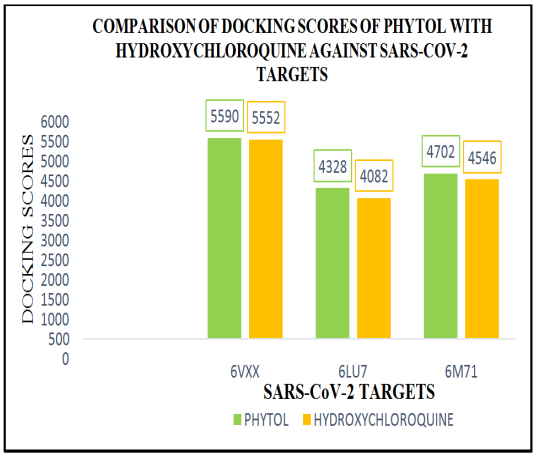

Figure 6: Comparison of phytol with hydroxychloroquine using docking score against SARS-CoV-2 targets (6VXX, 6LU7 and 6M71)

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REVIEW ARTICLE

An Overview of Siddha Herbal Preparation *Veppampattai kudineer* for Covid-19

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ABSTRACT

Siddha is one of the Indian traditional medical systems; the main concept of this system is prevention is better than cure. The number of positive patients with COVID infection and mortality also tremendously increased all over the world. Even after the vaccination the incidence of COVID infection not reduced. In this condition using of traditional preventive medicines against any kind of viral infection may help to reduce the COVID incidence and its burdens. Immunomodulator and anti-viral playing a vital role in the prevention and management of COVID. The present study is aimed to review the anti-viral, immunomodulator, anti-pyretic property of Neem bark (*Azadirachta indica*). It has several pharmacological actions without producing any adverse effects. In Siddha system of medicine it has been used as Veppam pattaikudineer, a herbal formulation which is indicated for fever, cold, cough, throat infections, kapha disease (respiratory disorders) and related conditions. The review results shows that the drug neem bark was scientifically proved for its anti-pyretic property, antiviral property against Newcastle Disease Virus and also has immune enhancing property due to the presence of phytoconstituents on neem bark. Moreover this *Veppam pattaikudineer* has been traditionally used for all kind of viral infections. Thus this study suggests that the *Veppampattai kudineer* may recommend as a prophylaxis and immune boosting medicine to manage this current COVID 19 pandemic.

Key words: Veppampattai kudineer; *Azhadirachta indica*; immunomodulator; COVID-19.

INTRODUCTION

According to WHO, as on August 2021, approximately 21,81,72,392people affected by Covid-19 in worldwide out of 3.27 cr in India confirmed cases including more than 5lakhs death. Coronavirus disease (covid- 19) is an infectious disease caused by the SARS-COV-2 virus most people who fall sick with covid -19 will experience mild to moderate symptoms and recover without special treatment. However some will become seriously ill and require medical attention [1]. Each and every country was affected by Covid-19. People around the world were affected directly and indirectly by it. The lockdowns imposed due to prevent the spread of Covid-19made the economies come to a standstill which lead to loss of jobs for millions of people leading to worldwide recession. Shortly after that they determined that the pneumonia was caused by virus which is of Zoonotic origin and it's caused by novel coronavirus. So there is an urgent need to find a drug or cure to stop the virus spread worldwide as no drug or cure has been found till now. So, nowadays the entire population depend upon traditional medicine and plants based therapeutics for the cost effective treatment and lesser adverse effects. In this view, in India traditionally the Neem bark has been used for various viral and other infectious diseases [2]. In order to promote the use of medicinal plants as potential source for immunomodulatory compounds. In this view, a bitter principle Nimbidin found in Neem bark was found to be antipyretic, non-irritant and effective [3] and also Neem has anti-viral, anti pyritic, anti-inflammatory, anti-oxidant, Immunomodulatory, anti-cancer, anti-ulcer, anti-bacterial, anti-malarial, anti-fungal and anti-tumoractivities. The different parts of Neem plant has been used to treat fever, diabetic, urinary disorder, fatigue, cough, vomiting, *Pitha* and *Kabha dosham*, loss of taste, skin diseases [4]. In Siddha system of medicine Neem is one of the *Kayakarpa* herbs (Rejuvenating herb) which possess antioxidant activity which means an elixir to maintain healthy life and used for non-communicable diseases (NCD) like diabetes, cancer, etc.

Veppampattai kudineer is an herbal Siddha preparation and used for intermittentfever, tiredness followed by fever [5]. This study is aimed to review the anti-viral, immunomodulator, anti-inflammatory activity of Neem bark through literature and scientific review.

Veppamattai (*Azadirachta indica*) Kudineer [5]:

Ingredients

Flesh of Neem bark	-	70 gm
Water	-	720 ml

From the above ingredients, the decoction is prepared by boiling it an hour to get indicated dosage level which is indicated for intermittent fever (*Murai suram*) with therapeutic dosage of 30-45ml.

The taste of bitter, astringent according to siddha text (Thotrakirama arachiyum siddha maruthuva varalarum) it is indicated for Throat and Kabha diseases. Neem has bitter, astringent and pungent taste and great therapeutic actions in various infectious diseases. As per Siddha literature, it has tonic, astringent, anti periodic and vermifuge actions [5]. The Siddha pharmacological characters are listed in Table.No:2. Neem bark has different phytoconstituents which are responsible for its anti-viral, anti-pyretic, anti-inflammatory, immunomodulatory and anti-tumor properties. (Table.No:3).

Phytochemicals

Surbhi singhal *et.al.* Isolated the phytochemicals from theNeem bark. It contains nimbin (0.042%), nimbidin (0.001%), nimbidin (0.4%), Nimbosterol(0.3%),alkaloids - 4.1%, flavonoids 2.5%crude glycosides 4.5%,crude glycosides 4.5%, cardiac glycosides 5.0%, steroid and triterpenoids 3.0%, tannic acid 0.643% and saponins 4.7%. All these compounds isolated from various parts of neem have various application in health care, pesticide and cosmetic industry [8]. Sunday E. et al,evaluated thephytoconstituents in neem (*Azadirachta indica)*by using techniques such as HPLC–MS, GC– MS, NMR and Infrared spectroscopy have revealed that phytocompounds like azadirachtins, nimocinol,

isomeldenin, azadirachtol (tetranortriterpernoid), 2,30-dehydrosalanol gedunin, nimbin, nimolicinol, odoratone, azadironolide, isoazadironolide, naheedin and mahmoodin are responsible for the various biological, pharmacological and toxicological properties [9]. Alok Maithani et.al isolated the compounds from neem stem bark*(A. indica)*such as gallic acid, (+) gallocatechin, (–)epicatechin, (+) catechin epigallocatechin, gallic acid, (–) epicatechin, catechinand diterpenoids, margolone, margolonone, isomargolonone are responsible for anti-inflammatory and anti-bacterial activity [10].

Immunomodulatory effect

Mohammad A,Alzohairy, et.al, performed the experiment to investigate the growth promoting and immunemodulatory effects of neem leaves(*A. indica)* infusion on broiler chicks and the results showed that neem infusion successfully improved antibody titre, growth performance, and gross return at the level of 50mL/liter of fresh drinking water. Another study investigated the effects of feeding of powdered dry leaves of *A. indica* (AI) on humoral and cell mediated immune responses in broilers and results showed that AI (2 g/kg) treatment significantly enhanced the antibody titres against new castle disease virus (NCDV)antigen [11]. Njiro,S.M.,et al. studied thatthe effect of an aqueous extract of *Azadirachta indica* on the immune response in mice, using the haemolytic plaque technique. An aqueous extract of *Azadirachta indica* stem bark was shown to enhance the immune response of BALB/C mice to sheep red blood cells *in vivo.* On the basis of these findings that it is concluded that, the aqueous extractof both stem bark and leaves of *A. indica* significantly enhances the immune response of mice againstSRBC, a thymus dependent antigen [12].

Ray et al, Modulation of humoral and cell mediated immune responses by *Azadirachta indica* in mice, the effect of *A. indica* (Neem) was evaluate on test of humoral and immune responses after 3 weeks oral leaf extract treatment in ovalbumin immunized mice. The dose level tested by 100mg/kg. The testes for humoralimmune response AI(100mg/kg) treated mice IgM and IgG levels. This test results are discussed in light of the possible immunopotentiating effects of AI [13].

Anti-oxidant activity

Amal kumar et al studied the Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica.* Anti-oxidant activity, the hexane fraction of bark showed the highest nimbin content (271 μg/g dw) followed by the methanolic extract (260 μg/g dw). Antioxidant activity was estimated by measuring 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity, hydroxyl radical scavenging activity, DNA protection assay, metal chelating and the reticence of peroxidation using linoleic acid system and their results were found at different measure of potency. The results of TP content expressed in tannic acid parallel ranged from 66.63 to 629.04 μg/mg in the bark extracts and 23.85 to 237.00 μg/mg in the leaf extracts. Likewise, the content of TF expressed in quercetine parallel ranged from 12.87 to 17.07 μg/mg in the bark and 13.72 to 93.17μg/mg in the leaf extract [14]. Bushra Sultana et.al reported the Anti-oxidant activity of phenolic components present in barks of *Azadirachta indica.* It is evaluated for its antioxidant activity, total phenolic (TP), and total flavonoids (TF) contents. Antioxidant activity was bound by metering reducing power, repression of peroxidation using linoleic acid system and 2,20-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity.The bark extract exhibited wide range of total phenolic,7.8–16.5 gallic acid equivalents and total flavonoid contents, 1.59–4.93 catechin equivalents. Reducing power at 10 mg/mL extract concentrationranged from 1.34 to 1.87. Different bark extracts to suppress the oxidation of linoleic acid by 44–90% while DPPH radical scavenging activity ranged from 49% to 87%.Finally it was found to offer the most efficient antioxidant activity [15].

M. Kiranmai et al, studied the free radical scavenging activity of *Azadirachta indica* root bark extract. In this study the extract was subjected to free radical scavenging activity by DPPH evaluation and total antioxidant activity. It is exhibited higher free radical scavenging effect on the DPPH evaluation with 50% scavenging activity at 27.3μg/ml and antioxidant activity was determined to be 0.58Mm of standard ascorpic acid [16].

Kiranmai, M. Et.al studied theAntioxidant activity and total flavonoids content of different parts of *Azadirachta indica* and investigated the antioxidant properties and total flavonoid contents of successive solvent extracts of different parts of AI.The total antioxidant assay was performed using the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant potential (FRAP) methods. From this study the total flavonoids content of different parts of *Azadirachta indica* and root bark methanolic extract showed significant antioxidant activities due to free radical scavenging potential [17]. Abdulaziz Rabiu Abdulkadir,et al, evaluated the *in-vitro* Antioxidant Potential in Leaf, Stem and Bark of *Azadirachta indica*.1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging and Ferric-Reducing Antioxidant Power Assay (FRAP) were used to estimate antioxidant activity the result of the study revealed that bark and leaf extract manifest higher free radical scavenging activity, with IC50 value of 23.27 and 55.07 (μg/ml) [18].

Anti - pyretic activity

M. Mahabub-Uz-Zaman et.al studied the Anti-inflammatory, Antinociceptive and Antipyretic activities of ethanol extract of *Azadirachta indica* Leaves. The activity was estimated on yeast-induced pyrexia in rats.Oral management of the ethanol extract of *A. indica* leaves significantly ($P<0.05$) suppressed the paw edema induced by carrageenan as well as granulomatous tissue formation induced by cotton pellet in rats at high dose level (1g/kg). A significant antipyretic effect ($P< 0.05$) was noticed with ethanol leaf extract of *A.indica* leaves at 1g/kg and 500 mg/kg dose level. In acute toxicity study, no mortality was observed at 4 g/kg dose level [19].

Anti-inflammatory activity

S. Manogaran et.al studied theanti – inflammatory and antimicrobial activitiesof the root, bark and leaves of *Azadirachta indica*. The paw volume was measured 0 hr, 1hr, 2hr after the injection of carrageenan (0.1 ml of 1% solution injected in the subplantar region.As a result,it showsthe histamine, and prostaglandin inhibitory activity. Among these root possessed profoundactivity. And also evaluated the activity of the root, bark and leaves of *A. indica onEscherichiacoli* of the Gram negative group. The very interesting featureobserved in antimicrobial activity was extracts havemore potent bactericidal activity thanstreptomycin [20].

Antimicrobial activity

Talha Bin Emran et.alstudied the phytochemical, antimicrobial, analgesic and anti-inflammatory properties of the ethanol extractand chloroform extract of *Azadirachta indica* for phytochemical study. The phytochemical screening of the three extracts of *Azadirachta indica* exhibited the presence of important secondary metabolites such as flavonoids, terpenoids, steroids and tannins.For the analgesic properties of ethanol extract of *Azadirachta indica* was studied by usinghot plate and acetic acid induced writhing methods. At two different doses (250 and 500 mg/kg body weight), the analgesic tests were performed on Swiss Albino mice. Also, the anti-inflammatory tests were performed by carrageenan induced paw edema method on long Evans rats at the two different doses of 250 and 500 mg/kg body weight using ethanol extract. Our results indicated that *Azadirachta indica* possesses remarkable analgesic and anti-inflammatory activity. The extracts showed potential antimicrobial activities against thirteen different strains of microorganisms like *Bacillus sereus,Bacillus subtilis, E-coli* etc [3].

Anti-viral activity

M. Shahid Mahmood et.al evaluated the antiviral activity of *Azadirachta indica* (Neem) bark extract against Newcastle Disease Virus.In this studyvarious dilutions of *Azadirachta indica* extract was used against NDV. *In-vitro* evaluation was done by performing spot assay and micro-hemagglutination test, while *in-vitro* antiviral activity was assessed by injecting the extracts in 11 days old embryonated eggs. During *in-vitro* evaluation, it was found that the Neem bark extract exhibit antiviral activity up to 1:4 dilution [21]. Vaibhav Tiwari et.al evaluated the *in-vitro* anti-viral activity of *Azaridacta indica* bark extract against herpes simplex virus Type 1 infection. The extract from Neem bark (NBE) significantly blocked HSV 1 infection into cells at concentration ranging from 50-100 μgm/ml. The cells treated with NBE also inhibited HSV- 1 glycoprotein mediated cells to cells fusion and polykaryocyts formation [22].

Shanmuga Subramanian et.al studied about that compounds from Neem leaves extract exhibit binding affinity as high as -14.3 kcal/mol against COVID-19 Main Protease (Mpro): A Molecular Docking Study, the binding energies obtained from the docking of 6LU7 with meliacinanhydride, nimocinol, isomeldenin, nimbolide, zafaral, nimbandiol, nimbin, nimbinene, desacetylnimbin were -14.3, -12.4, -12.3, -12.2, -11.9, -11.8, -11.7, -11.7, -11.4 kcal/mol respectively. This study suggests that Meliacinanhydride (Ki=33.36 pM) and the compounds from Neem leaves may be a potential treatment option against COVID-19 [2]. Badal et al, conducted a study on In-vitro anti-viral activity of Neem (*Azadirachta indica*). Leaf extracts against group B Coxsackieviruses.NCL 11 was studied regarding its activity and possible mechanism against Coxsackie group B viruses.NCL 11 inhibited plaque formation in 6 antigenic types of Coxsackie virus B at a concentration of 1000micrograms/ml at 96 hours [23].

Anti-bacterial

Faiza Aslam,et al carried out the study about antibacterial activity of various phytoconstituents of Neem. The phytoconstituent like alkaloids, steroids, tannins, glycosides, flavonoids and saponins and neem extract were applied against three bacterial strains i.e., *Staphylococcus areous, Corynebacterium bovi* and *E.coli* by using disc diffusion method. The inhibition zones were measured in millimeter with the help of a zone reader. The shows that the inhibition zones of neem extract were greater than each of the phytoconstituents [24].

CONCLUSIONS

It is considered as safety traditional plant and has several pharmacological actions, biological active components without any adverse effects. Siddha literature also describes the bitter taste has germicidal activity and neutralize the *kabha* derangement. This review study summarized the role of neem bark decoction*(Veppampattai kudineer)* in the prevention and treatment aspect of *kapha* diseases. Also ingesting orally will boost our immunity. Naturally occurring the neem bark has been already known for medicinal properties predominantly anti-oxidant, anti pyretic, anti-viral, immunomodulatory and anti-inflammatory activities. This review article provides the information about bark of neem tree used in *Veppampattai kudineer* from siddha literature. From the scientific evidences about the biological action and Phytochemicals present in the Neem bark, this single herbal formulation *Veppampattai kudineer* is safe and immunomodulatory remedial siddha medicine for Covid-19.

REFERENCES

1. WHO 2020,(COVID-19) Dashboard
2. Shanmuga Subramanian, Govt College of Engineering Tirunelveli, India. Email:ramanean@gmail.com;Twitter:Ramanean
3. Talha Bin Emran et al, Phytochemical, antimicrobial, cytotoxin, analgesic and anti- inflammatory properties of Azadirachta indica: A Therapeutic study, Bioanalysis and Biomedicine 2015,S12
4. Dr K.M. Nadkarni Indian materia medica, vol 1, A.K.Nadkarni popular prakashan.
5. Murugesa muthaliar K C., Gunapadam muthal vaguppu- Mooligai, Indian medicine and homeopathy, Seventh edition 2003.
6. The Siddha pharmacopeia if India Part 1, Vol 1, First edition, Govt of India ministry of health and family welfare, Department of AYUSH,Delhi.
7. Kausik Biswas et.al, Biological activities and medicinal properties of neem(*A.indica*), Current science, vol.82,no.11, June 2002.
8. Surbhi singhal and et.al, Review on a natural drugstore- neem, world journal of pharmacy and pharmaceutical sciences, 2016; 5(8): 379-396.
9. Sunday E et al,*Azhadirachta indica* (Neem):A plant of biological and pharmacological activites,Phytochem revise (2009) 8:601–620
10. Alok Maithani et al,*Azadirachta indica*(Neem) leaf :A review, Journal of pharmacy research,2011,4(6) 1824-1827

11. Mohammad A., The therapeutic role of *A.indica* (neem) and their active constituents in diseases prevention and treatment, Evidance – based complementary and alternative medicine, vol 2016.
12. NJIRO et al, Effect of an aqueous extract of *Azadirachta indica* on theimmune response in mice. *Onderstepoort Journal of Veterinary Research,* 66:59-62.
13. A Ray et al., Modulation of humoral and cell mediated immune response by *A.indica* (Neem) in mice, *Indian journal of experimental biology. Vol 34*, July 1996.
14. Amal Kumar et al, Anti-oxidant activity and quantitative estimation of azadirachtin and nimbin in a.indica. Juss grown in foothills of Nepal. African journal of biotechnology, vol 8(13).
15. Bushra Sultana et al, Anti-oxidant activity phenolic compounds presence in Bark of *A.indica, T. arjuna, A.nilotica* and Eugenia jambolina Lam.trees, Food chemistry 104(2007)1106-1114.
16. M.kiranmai et.al,Free radical scavenging activity of *Azadirachta indica* root bark extract Asian journal of pharmaceutical and clinical research 4(4),134-136;2011
17. Kiranmai, M Et al, , Antioxidant activity and total flavonoids content of different parts of *Azadirachta indica* A. Juss ., Journal of Medicinal Plants Research Vol. 6(46), pp. 5737-5742, 3 December, 2012
18. Abdulaziz Rabiu Abdulkadir et al, *in-vitro* Antioxidant Potential in Leaf, Stem and Bark of *Azadirachta indica,*Tropical agricultural science, 40 (4): 497 – 506 (2017)
19. M. Mahabub-Uz-Zaman et al, Studies on anti-inflammatory antinociceptive and anti pyritic activites of ethonal extract of *A. indica* leaves, Bangaladesh journal of scientific and industrial research 44(2)199-206,2009.
20. S. Manogaran et al, Ant-inflammatory and anti micriobial activities of the root bark and leaves of A.indica, ncient life of science 18(1)July 1998, pg 29-34.
21. M. Shahid Mahmood et al,Evaluation of antiviral activity of *Azadirachta indica* (Neem) bark extract against Newcastle Disease Virus,Pakistan veterinary journal 38(1):25-28,2018.
22. Vaibhav Tiwari Et.al, Invitro anti-viral activity of neem (*A.indica* L) bark extract against HSV type 1 infection, phytotherapy 24(8);1132-1140,2010.
23. Badal et al,In-vitro anti-viral activity of Neem (*Azadirachta indica*) Leaf extract against group B Coxsackieviruses, Journal of communicable diseases, 1 June 1999,31(2):79-90.
24. Faiza Aslam et al, Anti-bacterial activity of various phytoconstituents of Neem, Pakistan journal of agricultural science,vol 46(3), 2009.

Table. No: 1. **Siddha Pharmacological characters**

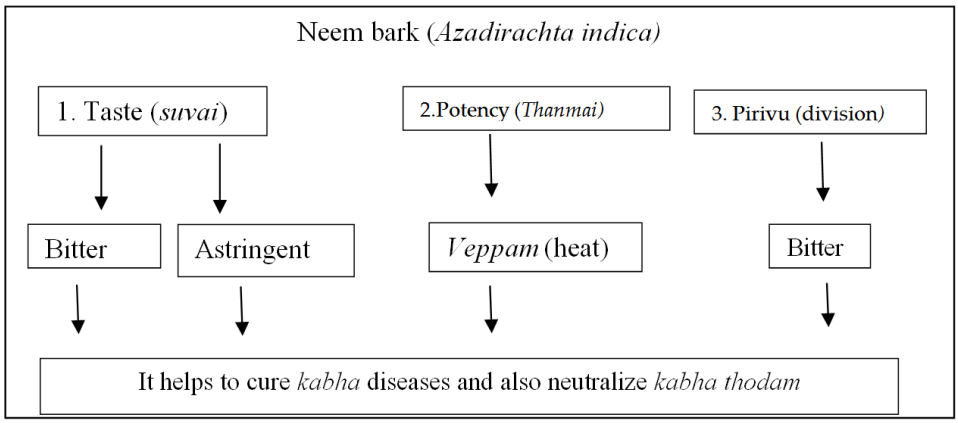

Table. No :2 Siddha Pharmacological characters of *Veppam pattai* Neem bark

Taste [5,6]	Part used [5]	Actions	Phytoconstituents [6]	Medicinal uses [5,6]
Bitter Astringent Pungent	Bark	Anti-viral, Anti periodic, Anti-inflammatory, Anti-oxidant, Anti-bacterial, Immunomodulatory	Nimbin, nimbinin, Nimbidin, sugiol, essential oil,gallic acid, epicatechin, catechin, tannins	Fever, Indigestion, peptic ulcer, constipation, worm infestations, vatha diseases, tiredness followed by fever.

Table. No: 3. Phytoconstituents and Pharmacologocal activity of Neem bark [7]

Phytoconstitutents	Pharmacological activity
Nimbidin	Anti-pyretic, Anti-viral,Anti-bacterial,Anti-inflammatory,Hypoglycemic,Anti gastric ulcer,Spermicidal,Diuretic, Anti-fungal
gallic acid, (-) picatechin	Anti-inflammatory, Immunomodulatory
Catechin, epicatechin	Anti-inflammatory, immune modulatory
NB2 peptidoglycan	immune modulatory
Polysachaidea G1A,G1B	Antitumor
Polysachaidea G1A,G1B	Anti inflammatory
Margolonone,isomargolonone and Margolone	Antibacterial

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RESEARCH ARTICLE

A Comparison Study of Pattern Recognition Classifiers for Printed Devanagari Characters

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ABSTRACT

In this research work, a comparison study of various pattern recognition classifiers and procedures which are used to identify the handwritten or printed Devanagari (Indian-script) characters and numeric values is held through OCR, Artificial Inelegancy (AI) and machine learning techniques. The recognition of handwritten characters (Indian-Lipi) by computer system is a complicated task, in current era. A number of research works related to the recognition of Hindi-Lipi based data have already been introduced in last three decades, although it is a very gigantic challenge for the recognition of handwritten based documents (Indian-script).OCR plays an important role to recognize the printed data materials of India script, today. In this study, we planned a research work for the recognition of Devanagari handwritten characters by different classifiers.

Keywords: Optical Character Recognition, Indian script, Features extraction, K-Means, Classifiers, Character classification.

INTRODUCTION

Devaganagri lipi is widely used for documentation and communication purpose in India. It is also known as Indian Script or Hindi-Script. It is treated as mother script among the Indian culture for various applications and uses. Devanagari lipi is mostly used for writing and reading the different data materials. The development of Devanagari language is originated from Brahmi lipi. Hindi-Script is officially used almost all nearby regions of India. In ancient time, Vedas and manuscripts, epics have been written manually for their exploration in all around the world. Devanagari script is very much easier for communication also [1-3]. Devanagari Script has a rich library of characters and numeric values for preparing the various documents and data, calculations. The handwritten documents of Hindi-lipi are easier to understand by human being but still difficult to recognize electronically through computer

system or any other computing device. A number of research people have implemented many experimental works to recognize the scanned or printed data of Devanagari lipi [4, 5].The different writing styles and methods made difficult to clarify or recognize the handwritten data (Hindi-Script) by the computer system in digital form. Optical Character Recognition (OCR) system helps to recognize the scanned or printed data items of Indian-Script in digital form easily with the help of various classifiers and techniques [6-8]. Here, in this work/ study, the performance of five dissimilar classifiers and features will be investigated / analyzed to recognize the modified data or images (gradient and curvature) as a result. The different classifiers like: Modified Quadratic Discriminant Function (MQDF), Linear Discriminant Function (LD), Support Vector Machine (SVM), Compound Projection Distance (CPD), Compound Modified Quadratic Discriminant Function (CMQDE) have been explored for this manuscript/ research work, here [9,10].In this section , the brief introduction of Devanagari script is discussed properly.

Devanagari (Indian Script)

Devanagari language is very well-known and famous language which is spoken in all nearby regions of India for many applications like: writing and reading the documents or records, communication purpose and data/reports creation/presentation. Devanagari lipi has a unique place among the Indian cultures. Devanagari language is widely used in other countries also [11]. The different books and articles, epics have already been written in Indian –Script (Hindi lipi). Devanagari script is also known as mother script or language of India. To recognize the handwritten data (Hindi lipi) by the computer system is a complicated job and interesting / demanding research field of human life. The research work related to recognition of data in various languages like: Nepali, Russian, Chinese etc is also difficult to be understood by digital devices and carried out with the help of various methods / technologies around the world [2]. Devanagari lipi consists of 49 primary alphabets, 13 vowels (Swars), and 36 consonants (Vyanjans), and 10 digits (Ankas) [12, 13]. Devanagari script or lipi is written from left-hand side to right-hand side series of alphabets. Following figures are providing a glance of Vowels and their Corresponding Modifiers, and Consonants in Hindi Scripts-

Feature Extraction

There are four sets used to recognize the data. These sets are divided into two types of sets separately for both types of images. First two sets are responsible to contain the image information (gradient) only and other two sets are required to calculate the curvature information with gradient image also. We used dataset based on grey-scale to get the binary features and these images were converted as in digital form using the method of “Otsu” [10, 14]. The length (parameters) for all sets have fixed at 392 dim. And the calculation techniques for feature sets have written below accordingly [9, 11]:

Calculation of gradient feature

A filtering (2x2) is being applied on the put in image (4 times) & normalization (non-sequential size) over the picture [11].The picture is tabulated by the pixels (148 x148) and separated with groups (49x49). With the help of pixels, the groups are derived in meaningful format having the value of A= (49/148) x (p-1)+1 and B = (49/148) x (q-1)+1. Here p and q are showing the dimensional manner for pixels (148x148) and A, B are showing the dimensional information for groups, individually. The gradient image derived from normalized image is produced by a filter named “Roberts”. After that, the slope of curve with tangent value is primarily segmented throughout the thirty-two ways with the gaps of /4x4 and the capacity of slope is calculated for every estimated way of situations. With the help of given formula, the capacity of slope/gradient can be measure:$SG = \sqrt{(\Delta u)^2 + (\Delta v)^2}$
& for the way of slope ($\theta(x, y)$) we mean$\theta(x, y) = tan^{-1} \frac{\Delta v}{\Delta u}$

At last, the groups (49x49) are reduced by small groups/blocks (7x7) with the help of filter (Gaussian) and repetitions have also reduced with eight routes, there after the dimensional feature vectors (7x7x8=392) are obtained [12].

Calculation of curvature feature

We calculated curvature feature with various ways and methods. The algorithm having many phases is written below to get the improved features [15];

1. The gradient's direction is divided to 32 levels.
2. The shape of curve is calculated by bi-quadratic and interpolation methods for three levels of divisions. (It is assumed t=0.15 for experimental work.)
3. The gradient's strength is gathered with thirty-two ways/routes, separately.
4. From thirty two routes, the sixteen routes/directions are filtered [1 4 6 4 1].In addition, Gaussian-filter provides the well ordered groups (7x7) from the big groups/blocks. With the help of curve's shape, the features are examined (3x392=1176 dim.)
5. The structural feature is reduced by 392 from 1179 spatial with the help of principal component analysis. It is using by the classifiers intended for evaluation and analysis of result.

About the Classifiers

The detailed information is written below to understand about the various five Classifiers (MQDF, LD, CPD, CMQDE and SVM) used here for image/offline data recognition purpose with applied work and processes:

The Modified Quadratic Discriminant Function (MQDF)

Modified quadratic discriminant function is defined as follows [13].

$$g(X) = (N + N_0 + n - 1)Ln\left[1 + \frac{1}{N_0\sigma^2}\left[\|X - M\|^2 - \sum_{i=1}^{k} \frac{\lambda_i}{\lambda_i + \frac{N_0}{N}\sigma^2}\left\{\varphi_i^T\,(X - M)\right\}^2\right]\right] + \sum_{i=1}^{k} \ln(\lambda_i + \frac{N_0}{N}\sigma^2)$$

where, X denotes the attribute vector of an enter character; M denotes a mean vector of trials or samples; φ_i^T indicates the ith eigen vector of the sample covariance matrix; λ_i indicates the ith eigen value of the sample covariance matrix; k shows the number of eigen values considered here, n is the feature size; σ^2 is the early inference of a variance; N is the digit of erudition samples; and N_0 is a assurance constant for σ.

The Linear Discriminant Function (LD): Linear discriminant function (LD) is defined by

$$g(X) = W^T X + W_0$$
$$W = S_W^{-1} M$$
$$W_0 = -\frac{1}{2}\, M^T\, S_W^{-1} M$$

Where , S_W denotes the covariance matrix within-class

Compound Projection Distance (CPD) and Compound Modified Quadratic Discriminant Function (CMQDF)

The projection space or distance provides a number of compound discriminant functions, the MQDF & more on to distinguish the comparable shaped puzzling character pairs efficiently for Devanagari character recognition [15]. The multiple projection space is an extensive projection space or length such that the dissimilarity between the signify vectors of the puzzling pairs are openly taken into clarification, and is defined as a linear grouping of the projection length or distance and the extension.

$$g_{cpd}^2\,(X) = (1 - \delta) g_{pd}^2\,(X) + \delta\, G_{cpd}^2\,(X)\,, (0 \le \delta \le 1)$$

The extension is defined by

$$G_{cpd}^2\,(X) = \frac{\left[M^T Y - \sum_{i=1}^{k} \{M^T \varphi_i\}\{Y^T \varphi_i\}\,\right]^2}{M^T M - \sum_{i=1}^{k} \{M^T \varphi_i\}^2}$$

$$M = M_2 - M_1, Y = X - M_1$$

The Support Vector Machine (SVM)

An SVM is applied to define the problems of two-classes. It provides the best possible region that can increase the space for the nearby samples of classes, known as SVs. The collected data (N) for training purpose is given :{xn| n=1,...,N}.The formula is used for the same as follows:

$$f(x) = \sum_j \alpha_j x_j . x + b$$

Where $\{x_j\}$ denotes about the groups of support vectors, both parameters: b & αj have been determined by the solution of a problem (quadratic) [16].With the replacement of inner product can be treated as the non-linear classifier which may be upgraded to the linear SVM. The master method is defined as: A (a, b) =φ(a)xφ(b). It must clarify the condition of Mercer's [16, 17].

Classification Principle

Clustering is considered as a finest method for better data analysis in the field of database structure management system. A huge amount of data can be separated into small subgroups. Same valued/natured data must be stored in same sub groups. The identification task/process can be easily complete by this technique (clustering). Firstly, the data point can check the subparts that are similar in manners or types. The distance (Euclidean/ correlation) is measured to categorize this techniques. According to the sub grouping, the features of this specified application can be analyzed by clustering technique [4, 18]. This technique is treated as an unsupervised method based on machine learning (ML) for many analytical purposes.CNN (Convolutional neural network) provides the division of dataset features. Every separation of dataset is treated as clusters which are to be non-overlapped. All points with the features are closed to a single group only. We have to fix the number of clusters. The casual data point which is calculated, positioned as centroid and if the middle points are not fixed at proper centroid, it must be frequently held to reposition the data points again. Same cluster can have data points. It is to be measured the total length between data points and all centroids.

$$j = \sum_{i=1}^{m} \sum_{k=1}^{K} w_{ik} \left\| x^i - \mu_k \right\|^2$$

After that call SVM algorithm to evaluate k number of clusters. Sort number is denoted as a T. create a condition where every value can be evaluate as a newly generated solution. Then it will give kSVM solution.

ksvm-model = {(c1,Lsvm1), (c2,Lsvm2)...(ck,Lsvmk)};

Where, k= local model= no of cluster;

y=it is presented as parameter which is hyper of kernel function of RBF;

c= the error rate of SVM.

And in the very last return the global best solution. Repeat till all such cluster is pruned. And it gives final classification. Then we can classify and identify the characters and numeric values very efficiently. And calculate the accuracy of the characters recognition [19, 20]. In the fields of Machine Learning, perhaps K-Means is the most branded and studied method for clustering analysis [20-28]. K-means is a technique of clustering which helps to feed up new scanned data or images into required form of blocks for handwritten character categories. Using a desktop GUI form / web portal, a client can demand and visualize the historical images with the past gathered data from the server [29-35].

DISCUSSION AND RESULTS

Data is gathered and integrated from different resources for the experimental research work, here. We practiced 36143 samples of the primary characters (Hindi lipi) for the creation of dataset. This dataset is divided into 6 sub-sets for training reason and the residual sub-sets are used for matching/testing procedure of experimental work or study. The complete tests have produced the average accuracy rate of recognition, with the help of five classifiers used here. The results are measured or intended for couple of images or pictures which is clearly shown in Table 1. According to the above results, it is clearly analysis that Compound Projection Distance (CPD) classifier formed highest average accuracy (94.65%) rate of results for the data acknowledgment . And rest of classifiers has produced the less accuracy rate to recognize the data for both images / pictures. The LD classifier has given the lowest average accuracy (87.88%) rate for data recognition and acknowledgment among all the used five classifiers. As per result, it is originated that the curving feature shaped a high rate of accuracy outcomes than gradient features by all remained classifiers except MQDF, SVM, LD and CMQDE. The graph shows the results by various classifiers clearly in figure 8

CONCLUSION AND FUTURE SCOPE

In this manuscript, we considered five different classifiers to recognize the handwritten or printed characters of Indian script (Devanagari lipi). It provides a new way for future related advance research work in the area of pattern recognition and It is noted that the CPD (Compound Projection Distance) classifier has given the best recognition result of images with higher average accuracy rate (94.65%) among the different classifiers (MQDF, SVM, LD and CMQDE). This research work or comparison study will be encouraging for advance study work in the area of recognition of different languages based printed data items in future and it will be beneficial for all research sectors of data recognition.

REFERENCES

1. Plamondon R and Srihari S. N (2000) On-Line and off-line handwritten recognition: Acomprehensive survey. IEEE Trans on PAMI, Vol.22, pp.62-84.
2. Pal U and Chaudhuri B. B (2004) Indian script character recognition: A Survey Pattern Recognition.Vol. 37, pp. 1887-1899.
3. Liu C. L and Suen C. Y (2008) A new benchmark on the recognition of handwritten Bangla and Farshi numeral characters. In Proc. 11th ICFHR.
4. Sethi I. K and Chatterjee B (1977) Machine Recognition of constrained Hand printed Devnagari: Pattern Recognition.Vol. 9, pp. 69-75.
5. Pal U, Wakabayashi T, Sharma N and Kimura F (2007) Handwritten Recognition Numeral of Six Popular Indian Scripts. In Proc. 9th ICDAR, pp. 749-753.
6. Hanmandlu M and Murthy O. V. R (2005) Fuzzy Model Based Recognition Handwritten of Hindi Numerals. In Proc. Intl. Conf. on Cognition and Recognition, pp. 490-496.
7. Kumar S and Singh C (2005) A Study of Zernike Moments and its use in Devnagari Handwritten Character Recognition. In Proc. Intl. Conf. on Cognition and Recognition, pp.514-520.
8. Sharma N, Pal U, Kimura F and Pal S (2006) Recognition of Offline Handwritten Devnagari Characters using Quadratic Classifier. In Proc. Indian Conference on Computer Vision Graphics and Image Processing, pp- 805-816.
9. Pal U, Sharma N, Wakabayashi T and Kimura F (2007) Off- Line Handwritten Character Recognition of Devnagari Script. In Proc. 9th ICDAR, pp.496-500.
10. Otsu N (1979) A Threshold selection method from grey level histogram. IEEE Transon SMC, Vol.9, pp.62-66.

11. Kimura F, Wakabayashi T, Tsuruoka S and Miyake Y (1997) Improvement of handwritten Japanese character recognition using weighted direction code histogram: Pattern Recognition.Vol.30, No.8, pp.1329-1337.
12. Shi M, Fujisawa Y, Wakabayashi T and Kimura F (2000) Handwritten numeral recognition using gradient and curvature of gray scale images: Pattern Recognition.Vol.35, pp.2051- 2059.
13. Kimura F, Takashina K, Tsuruoka S and Miyake Y (1987) Modified quadratic discriminant function and the application to Chinese character recognition. IEEE Trans. on PAMI, Vol.9, pp 149-153.
14. Wakabayashi T, Shi M, Ohyama W and Kimura F (2002) A Comparative Study on Mirror Image Learning and ALSM. In Proc. 8th IWFHR, pp. 151-156.
15. Nakajima T, Wakabayashi T, Kimura F and Miyake Y (2000) Accuracy Improvement by Compound Discriminant Function for Resembling Character Recognition in Japanese. Trans. of IEICE, Vol.J83-D-II, pp.623-633.
16. Vapnik V (1995) The Nature of Statistical Learning Theory. Springer Verlang.
17. Pal U, Chanda S, Wakabayashi T and Kimura F (2008) Accuracy Improvement of Devnagari Character Recognition Combining SVM and MQDF. In Proc. 11th ICFHR, pp.367-372.
18. Meng S (2002) Study on Accuracy Improvement of Handwritten Character Recognition. Ph.D.Thesis, Mie University.
19. Nafiz Arica N (2000) An Overview of Character Recognition Focused On off-line Handwriting. C99-06-C-203, IEEE.
20. Cardona George and Jain Danesh (2003) The Indo-Aryan Languages. Routledge, ISBN 978- 0415772945, pages 68-69.
21. Singh Harikesh and Sharma R. K (2007) Moment in Online Handwritten Character Recognition. National Conference on Challenges & Opportunities in Information Technology ,Gobindgarh.
22. Hanmandlu M and Ramana Murthy O.V (2007) Fuzzy model based recognition of handwritten numerals, pattern recognition. vol.40, pp.1840-1854.
23. Ramteke R. J, Mehrotra S. C (2008) Recognition of Handwritten Devnagari Numerals. International Journal of Computer Processing of Oriental Languages.
24. Das, N, Basu, S, Sarkar, R, Kundu, M, Nasipuri, M, Basu, D (2009) Handwritten bangle compound character recognition: Potential challenges and probable solution. IICAI:1901-1913.
25. Das N, Das B, Sarkar R and Basu S (2010) Handwritten BanglaBasic and Compound character recognition using MLP and SVM classifier. Journal of Computing, Vol. 2, 2:109-115.
26. Bag S, Bhowmick P and Harit G (2011) Recognition of bengali handwritten characters using skeletal convexity and dynamic programming in Emerging Applications of Information Technology (EAIT). Second International Conference: 265–268.
27. Khan PathanImaran , Ahmed Bari Ahmed Ali Abdulbari and Ramteke R.J (2012) Recogni-tion of offline handwritten isolated Urdu character. International Journal on Advances in Computational Research. Vol. 4.Issue1:117-121.
28. Vaidya S A and Bombade B R (2013) A Novel Approach of Handwritten CharacterRecognition using Positional Feature Extraction. International Journal of computer science and Mobile Computing. Vol. 2, 6:179-186.
29. Wu, X, Tang, Y, and Bu W (2014) Offline Text-Independent Writer Identification Based on Scale Invariant Feature Transform. IEEE Transactions on Information Forensics and Security, 9, :526-536.
30. Dholakia K (2015) A Survey on Handwritten Character Recognition Techniques for Indian Languages, International Journal of Computer Applications. 115(1): 17-21.
31. Das N, Sarkar R, Basu S, Saha P.K., Kundu M and Nasipuri M (2015) Handwritten bangla character recognition using a soft computing paradigm embedded in two pass approach: Pattern Recognition . 48:2054-2071.
32. Sarkhel R, Das N, Saha A K and Nasipuri M (2016) A multi-objective approach towards cost effective isolated handwritten bangla character and digit recognition. Pattern Recognition. 58:172–189.
33. Singh R and Shukla A K (2018) A Literature Review on Handwritten Character Recognition based on Artificial Neural Network: 753-758.
34. Indian A (2017) A Survey of Offline Handwritten Hindi Character Recognition, 978-15090-6403-8/17. IEEE.

35. Shukla A.K (2020) Patient Diabetes Forecasting Based on Machine Learning Approach In: Pant M., Kumar Sharma T., Arya R., Sahana B., Zolfagharinia H. (eds) Soft Computing: Theories and Applications. Advances in Intelligent Systems and Computing. Vol 1154. Springer.Singapore.https://doi.org/10.1007/978-981-15-4032-5_91.

Table 1: Analysis of results by different classifier

Classifier	Grey image		Binary image		Average (%)
	Gradient (392 dim.)	Curvature (392 dim.)	Gradient (392 dim.)	Curvature (392 dim.)	
MQDF	94.27	94.79	94.16	94.55	94.44
LD	86.79	89.06	86.80	88.89	87.88
SVM	93.39	94.55	93.61	94.38	93.98
CPD	94.47	94.98	94.39	94.77	94.65
CMQDE	94.41	94.95	94.36	94.65	94.59

Figure 1:-Swars-Devanagri-Lipi

Figure 2:-Vyanjan- Devanagri-Lipi

Figure 3:- "Swars with Modifiers in Hindi-Script"

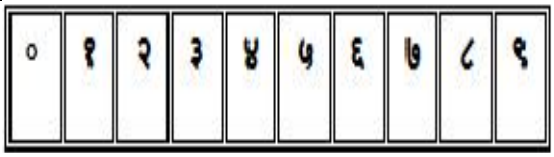


Figure 4:- "Anka in Indian-Script

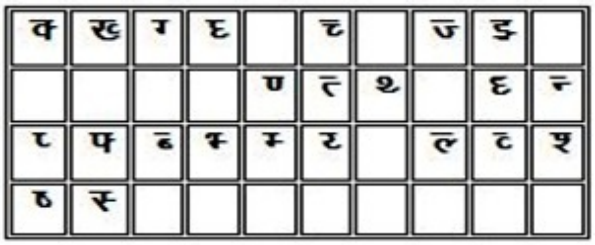


Figure 5:- "Semi Form of Vyanjans"

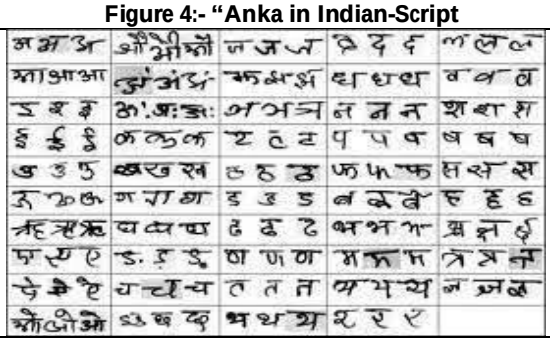
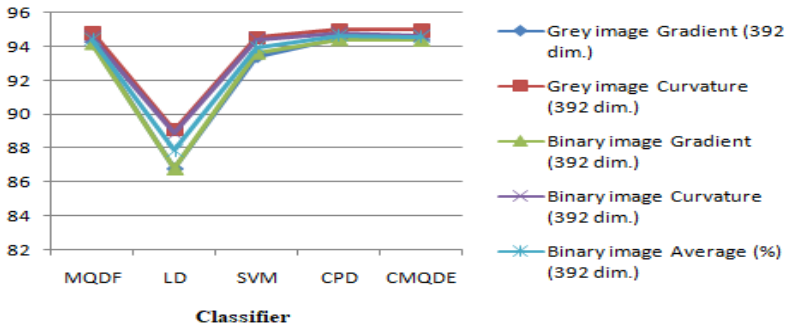


Figure 6: Printed Devanagari handwritten characters in same manner

$$j = \sum_{i=1}^{m}\sum_{k=1}^{K} w_{ik}\left\|x^i - \mu_k\right\|^2$$

Figure 7:- SVM Calculation



Graphical representation of results obtained from different classifiers

Figure 8: Graphical view of results by various classifiers

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RESEARCH ARTICLE

Evaluating the Efficacy of *Chlorococcum* in the Phycoremediation of HeavyMetals Contaminated Leather Industrial Wastewater

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ABSTRACT

Today, environmental pollution has negative consequences for humans and animals and is a major concern around the world. Effluent from the tannery sector is one of the most significant sources of pollution in the world. Most of the contaminants in the tannery sector are soluble in waste effluent which cannot be entirely eliminated using conventional methods. The goal of this research was to figure out the pollution load in an industrial effluent located near pallavaram vicinity in Chennai city. To meet the current scenario, a novel phycoremediation technology employing *Chlorococcum* species (green microalgae) to clean tannery effluent has been developed. The findings revealed that *Chlorococcum* species were highly successful in thephycoremediation process of wastewater treatment with nine heavy metals showing below reduction limits (Se, Cd, Cu, Pb, Hg, Cr, As and Sn). Microalgae's capacity to absorb heavy metals polluting the environment is studied. Phycoremediation is cheaper, easier, greener alternative in the field of water treatment and more secure for the environment.

Keywords: BOD, Effluent, Heavy metals, *Chlorococcum*, Phycoremediation, Microalgae.

INTRODUCTION

Anthropogenic activities like agricultural practices and industrialization, have posed a major danger to the aquatic and terrestrial biomes. Tannery industry wastewaters are a major cause of pollution in the environment which are toxic to both flora and fauna as well as to human health [1] [2]. Arsenic, lead, Cadmium, Copper, mercury,

Chromium , zinc, nickel and selenium are trace heavy metals found in tannery effluent which are capable of harming the environment. Unlike organic pollutants, heavy metals are not biodegradable, and pose a serious threat to living beings and the environment due to their carcinogenic and mutagenic properties [3]. Traditional metal removal processes are either becoming ineffective or growing more expensive as regulatory effluent limitations become more severe. As a result, proper treatment prior to final discharge into various aquatic environments is effective in minimising all negative repercussions.To remediate tanning industry wastewater, physical, chemical and biological [4] [5] approaches has been employed. As a result, demand for alternative, cost-effective solutions is significant. To reduce the water pollution, number of methods have been tried [6][7]. Among all attempts bioremediation has been proven to be an ecofriendly and efficient method for removing the toxic wastewater contaminants.

On the other hand, studies based on phycoremediation in wastewater treatment have been developed [8] [9]. Phycoremediation is the employment of microalgae or macroalgae to remove or biotransform polluting substances from wastewater, such as nutrients and xenobiotics [10] . Microalgae are being most successfully used in wastewater heavy metal removal because the photosynthetic algal strains has power to rectify harmful heavy metals concentrations and other contaminants. Autotrophic algae have a wide surface area, which allows them to consume a considerable pollutants from wastewater [11]. Mechanism of algae can decontaminate heavy metals from the effluent. The purpose of this present research is to analysis the physio-chemical parameters of tannery industry wastewater, as well as heavy metals toxicity and to compare it with the National Environmental Quality Standards **[12]** (NEQS) after phycoremediation with the microalgae (*Chlorococcum*).

MATERIALS AND METHODS

Sample Analysis

The pH of the sample was analyzed in the collection site itself with the help of digital pH meter. The sample effluent was tested for physio-chemical quantity prior to and after phycoremediation methods of treatment prescribed by APHA [13]. Microalgal cells were isolated before being analysed in the treated effluent using centrifugation.

Sample collection and algal isolation

Samples were collected from various locations along the Chromepet river.Water samples were collected using the Random Sampling Method (RSM) [14]. The collected samples were inoculated in BBM culture medium. The composition of the medium is given in Table 1. Cultureof *Chlorococcum* cultures were isolated using streak plate technique and serial dilution [15] [16]. [17].The selected strain *Chlorococcum* was employed in the tannery wastewater treatment experiment, the raw effluent was used as a control after feasibility test.

RESULTS AND DISCUSSION

Laboratory analysis of tannery industrial effluent sample was performed before and after phycoremediation, and the results showed that all wastewater parameters in the raw effluent werehigher than NEQS before treatment, and the results after treating with green algae (*Chlorococcum*) showed below the reduction limit and were much lower than NEQS .The treatment of effluents generated during tannery manufacture was investigated. Before and after phycoremediation with, the physio-chemical parameters were analysed. The microalgae has the capacity to lower Total Dissolved Solids (TDS) and to adjust p^H (Figure 1). The untreated effluent had high amount of Chemical Oxygen Demand and Biological Oxygen Demand of about 1670 and 6655 mg/L . In addition, Chloride, Sulphate and Chloride (Figure 4), TSS and TDS were also high (Figure 2). . On the other hand the effluent treated with *Chlorococcum* species have neutralized the acidity (pH) of the effluent [18]. The microalgae treated effluent had high minimization in the COD and BOD of about 15 and 65 mg/L, respectively (Figure 3). Noorjahan [19] observed high amount of BOD (600-1622 mg/L) in textile wastewater. Tannery effluent has also been shown to contain high

amounts of BOD [20]. The untreated wastewater had higher COD levels (2749.8 mg/L) than the treated effluent [21]. The treatment with microalgal resulted in a considerable reduction in the amount 22% BOD and 38% COD levels respectively [22].

Heavy metal uptake

Heavy metal parameters were assessed and the adsorption capability of *Chlorococcum* species was analyzed. The highest concentration of chromium was found to be 31.15 mg/L, followed by nickel, zinc, and lead of 0.47 , 0.28 , and 0.27 mg/L, respectively. Highest Cr concentration was found chrome-tanning effluents picking process (2075 mg/L) [23]. All the parameters were analyzed and compared to the values estimated by NEQS (National Environmental Quality Standards) (Table 2).

CONCLUSION

The findings of the investigation show that the tannery's effluent does not satisfy the legal ranges of selected parameters. Since tannery industrial effluent has a extensive range of biological, physical and chemical properties, designing an effective and suitable biological treatment process can be difficult. Algal treatment is a cost effective and efficient way to reduce pollutants from industrial effluent. High temperature in hot summer harmed algal growth, especially in an effluent with a high content of industrial wastewater. The phycoremediation procedure was determined to be a cost-effective treatment approach for tannery industry wastewater in the current study because it did not require any chemicals, energy, or power, making it economical

REFERENCES

1. S. Ben Younes, Z. Bouallagui, A. Gargoubi and S. Sayadi. "Investigation of Dyes Degradation Intermediates with *Scytalidium thermophilum* laccase". *Eur Food Res Technol* . Vol: 233, pp 751–758. 2011.
2. R.G. Saratale, G.D.Saratale, J.S. Chang and S.P. Govindwar. Ecofriendly Degradation of Sulfonated Diazo Dye C.I. Reactive Green 19A using *Micrococcus glutamicus* NCIM–2168. *Bioresour Technol* ,Vol 100, pp 3897–3905. 2009. DOI: 10.1016/j.biortech.2009.03.051.
3. W.Wu, P.Wu, F.Yang, D.Sun, D.Zhang, Yi and Zhou. Assessment of Heavy Metal Pollutionand Human Health Risks in Urban Soils Around an Electronics Manufacturing Facility. *Science of the Total Environment*, Vol 630, pp 53–61. 2018. DOI: 10.1016/j.scitotenv.2018.02.183
4. G. Durai and M. Rajasimman. Biological Treatment of Tannery Wastewater - *A review. Journal of Environmental Science and Technology*, Vol 4, pp 1–17. 2011. Doi :10.3923/jest.2011.1.17.
5. G. Lofrano , S. Meriç, G.E. Zengin and D. Orhon. Chemical and Biological Treatment Technologies for Leather Tannery Chemicals and Wastewaters: *A Review: Science of the Total Environment*, pp 265–281. 2013. Doi: 10.1016/j.scitotenv.2013.05.004
6. J. Dasgupta , J. Sikder, S. Chakraborty, S. Curcio and E. Drioli . "Remediation of TextileEffluents by Membrane Based Treatment Techniques: A State of the Art Review," *Journal of Environmental Management*, Vol 147, pp 55–72. 2015.
7. Madhavan J. Theerthagiri J. Balaji, D. Sunitha Choi S. and Ashokkumar M. Hybrid advanced oxidation processes involving ultrasound: An overview; Molecules. Vol 24(18),pp 3341. 2019.
8. I.C. Woertz, Lipid Productivity of Algae Grown on Dairy Wastewater as aPossible Feedstock for Biodiesel. California Polytechnic University, San Luis Obispo, 2007.
9. Y.S.Yun, S.B.Lee, J.M.Park, C.I. Lee and J.W.Yang. Carbon Dioxide Fixation by Algal Cultivation using Wastewater Nutrients. *J Chem Technol Biotechnol,* Vol 69(4), pp 451–455. 1997.
10. E.J.Olguín 2003. Phycoremediation: Key Issues for Cost-Effective Nutrient Removal Processes. *Biotechnology Advances.* Vol 22 , pp 81-91. Doi: 10.1016/s0734-9750(03)00130-7.

11. C.X. Hua, S.J. Traina, T.J. Logan, T. Gustafson, R.T. Sayreand and X..H. Cai . Applicationsof Eukaryotic Algae for the Removal of Heavy Metals from Water. *Molecular Marine Biology and Biotechnology*, Vol 4, pp 338-344. 1995.
12. National Environmental Quality Standards for municipal and liquid industrial effluents (NEQS). 2011. Url: http:// www.emc.com.pk/pdf/3-NEQS (New) .
13. APHA. Standard methods for the examination of water and wastewater. American Public Health Association, American Water Works Association, *Water Environment Federation*. Washington D.C. Vol 1360(23), pp 1546. 2017.
14. V. Uma Rani , S. Palanivel and U. Elayaperumal. Algal Diversity of Arulmigu SriThiyagarajaswamy Temple Tank Thiruvottiyur, Chennai, Tamil Nadu, India . *Indian Journal of Science and Technology*, Vol 10(13), pp 1-7. 2017. DOI: 10.17485/ijst/2017/v10i13/102203.
15. R.A. Andersen. 2005. Algal Culturing Technique. Salt Lake City: *Elseiver Academic Press*.
16. P. Pachiappan , B.B. Prasath, S. Perumal , S. Ananth , A. Shenbaga Devi, S.D. Kumar and
17. S. Jeyanthi. Isolation and Culture of Microalgae . Advances in Marine and Brackish water Aquaculture, pp 1–15. 2015. Doi:10.1007/978-81-322-2271-2_1.
18. D. Saranya and S. Shanthakumar. Green Microalgae for Combined Sewage and Tannery Effluent Treatment: Performance and Lipid Accumulation Potential. *Journal of Environmental Management*. Vol 241, pp 167–178. 2019. Doi:10.1016/j.jenvman.2019.04.031.
19. V. Sivasubramanian, V.V.Subramanian, B.G. Raghavan and R. Ranjithkumar. Large scale Phycoremediation of Acidic Effluent from an Alginate Industry. *Science*. Asia, Vol 35(3), pp 220-226. 2009. DOI: 10.2306/scienceasial513-1874.2009.35.220.
20. C.M.Noorjahan. Physicochemical Characteristics, Identification of Fungi and Biodegradation of Industrial Effluent. *Journal of Environmental and Earth Sciences*. ISSN 2225-0948 (Online). 4(4): 32 39. 2014.
21. R.T. Kulkarni Source and Characteristics of Dairy Wastes from a Medium Sized Effluent on Microorganisms, Plant Growth and their Microbial Change. *Advances in Life Sciences*, Vol 3,pp 76 - 86. 1992.
22. Arasappan Sugasini and Kalyanaraman Rajagopal. Characterization of Physicochemical Parameters and Heavy Metal Analysis of Tannery Effluent. *International Journal of Current Microbiology and applied sciences*. ISSN: 2319 – 7706, Vol 4(9), pp 349-359 , 2015. https://www.researchgate.net/publication/282842698.
23. P. Hanumantha Rao, R. Ranjith Kumar, B.G. Raghavan, V.V. Subramanian and V.Sivasubramanian. Application of Phycoremediation Technology in the Treatment of Wastewater from a Leather-Processing Chemical Manufacturing Facility. *Water* SA, Vol 37(1), pp 7 -14. 2011. ISSN 1816-7950 (On-line).http://www.wrc.org.za.
24. Manjushree Chowdhury., Mostafa M.G., Tapan Kumar Biswas, Abul Mandal and Ananda Kumar Saha. Characterization of the Effluents from Leather Processing Industries. Environmental Processes. *Springer International Publishing* Switzerland.2:173-187. 2015. Doi 10.1007/s40710-015-0065-7

Table 1: Bold's Basal Medium (BBM) Composition

MEDIA COMPONENT	STOCK SOLUTION	CULTURE MEDIUM (1 litre)
$NaNO_3$	5 g/200 ml	10 ml
$MgSO_4.7H_2O$	1.5 g/200 ml	10 ml
K_2HPO_4	1.5 g/200 ml	10 ml
KH_2PO_4	3.5 g/200 ml	10 ml
NaCl	0.5 g/200 ml	10 ml
$CaCl_2.2H_2O$	0.5 g/200 ml	10 ml
EDTA-KOH solution		1 ml
EDTA stock	5 g/100 ml	-
KOH	3.1 g/100 ml	-
Acidified Iron Solution		1 ml
$FeSO_4$ x $7H_2O$	0.498 g/100 ml	-
H_2SO_4		1 ml
Boron Solution		
H_3BO_3	1.14 g/100 ml	1 ml
Trace elements solution		1 ml
$CuSO_4.5H_2O$	1.57 g/L	-
$ZnSO_4.7H_2O$	8.82 g/L	-
$MnCl_2.4H_2O$	1.44 g/L	-
$Co(NO_3)_2.6H_2O$	0.49 g/L	-
MoO_3	0.71 g/L	-

Table 2: Comparison of heavy metals in tannery effluent (before and after phycoremediation) to National Environmental Quality Standards (NEQS , 2011)

S. No.	PARAMETERS	PRE-TREATMENT (mg/L)	POST TREATMENT (mg/L)	NEQS (2011) (mg/L)
1	Arsenic as As	BDL(DL:0.05)	BDL(DL:0.05)	1.0
2	Cadmium as Cd	BDL(DL:0.01)	BDL(DL:0.01)	0.1
3	Total Chromium as Cr	37.15	BDL(DL:0.1)	1.0
4	Copper as Cu	0.20	BDL(DL:0.12)	1.0
5	Lead as Pb	0.27	BDL(DL:0.01)	0.5
6	Mercury as Hg	BDL(DL:0.001)	BDL(DL:0.001)	0.01
7	Nickel as Ni	0.47	BDL(DL:0.1)	1.0
8	Selenium as Se	BDL(DL:0.01)	BDL(DL:0.01)	0.5
9	Zinc as Zn	0.28	0.05	5.0

BDL – Below Detection Limit; DL – Detection Limit

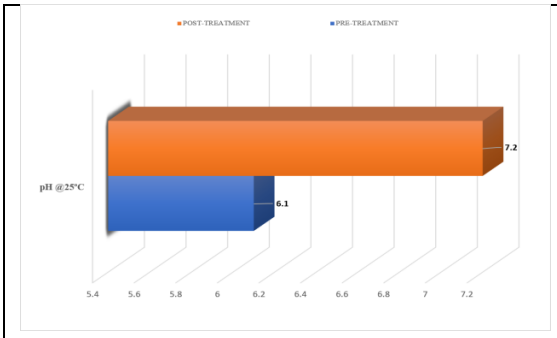

Figure 1: P^{H} of tannery effluent before and after phycoremediation

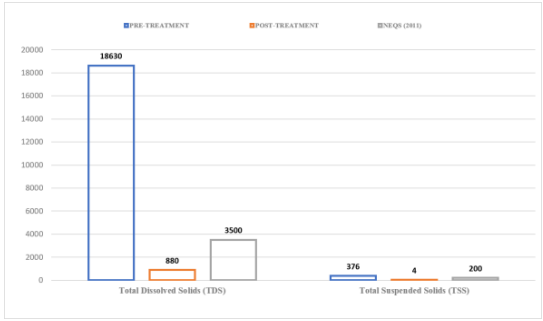

Figure 2: TDS and TSS values in tannery effluent (before and after phycoremediation) are compared to National Environmental Quality Standards (NEQS , 2011)

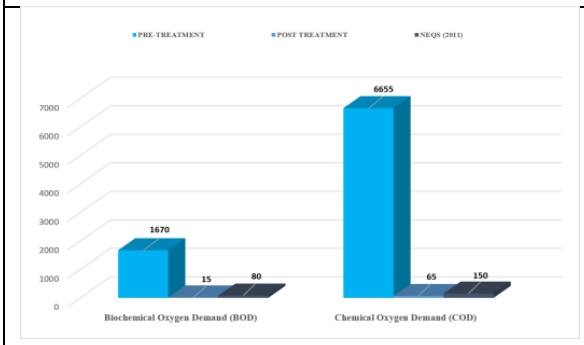

Figure 3: BOD and COD in tannery effluent (before and after phycoremediation) are compared to National Environmental QualityStandards (NEQS , 2011)

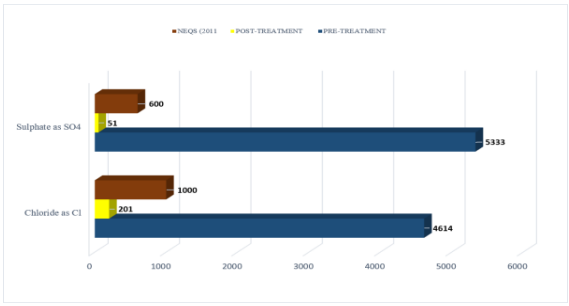

Figure 4: Sulphate and Chloride in tannery effluent (before and after phycoremediation) are compared to National Environmental Quality Standards (NEQS , 2011)

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REVIEW ARTICLE

A Short Overview on Gram-Negative Bacteria: *Salmonella typhimurium* and *Escherichia coli*

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ABSTRACT

Many of the Gram-negative bacteria such as: *Salmonella typhimurium* and *Escherichia coli*, are known from decades that cause life-threating diseases in human and many other animals. These bacteria are known to survive in the host body very quickly due to the presence of protective cell wall which defends from invasion of exogenous toxic agent. Therefore, understanding the underlying mechanism of action of these bacterial is of paramount important which will help in developing new molecular scaffold with appropriate architectural unit that can circumvent the preventive cell wall of such bacteria. Herein, we have covered the recent scientific development of these bacteria and their potential inhibitors against theselife-threating pathogenic Gram-negative bacteria. This brief review on inhibitors against gram-negative bacteria may open up a door way to design highly specific inhibitors with high efficacy against the pathogenic gram-negative bacteria.

Keywords: Gram-negative bacteria, *Salmonella typhimurium, Escherichia coli,* infected diseases, antibacterial activity, small organic molecule, pathogenic bacteria.

INTRODUCTION

Generally, bacteria are a variety of biological cell consisting of vast domain of single cell organism [1]. Bacteria having different type of shape such as rod, spiral and spheres. Bacteria colonize in soil, hot spring, acidic water, radioactive waste water, and also found in earth's crust. The study of structural and functional unit of bacterial cell is

called as bacteriology. There are more than billion bacteria present on earth but only 27% are discovered till now [2]. Directly or indirectly, all living organisms depend on bacteria as some bacteria and some archaea contain a specific gene which synthesize the vitamin B12 /Cyanocobalamin. Generally, living organisms consume it through food. Vitamin B12 is responsible for vitamin metabolism in a living cell. It is also responsible for DNA synthesis & metabolism of amino acid and carboxylic acid [3]. Bacteria and humans have several necessary relationships for which microorganisms make our lives easier through lots of ways. Typically, 1gram of soil contains nearly 40 million of bacteria and 1mL of water contains nearly 100000 of bacteria. Bacteria plays a vital role in carbon and nitrogen cycle. Biomass present in the earth mainly consists of carbon and nitrogen which is decomposed by the bacteria and release them to environment for taking part in carbon and nitrogen cycle. Most of the bacteria are harmless but some of them cause various diseases like tetanus, cough and syphilis and many more disease. The disease is transmitted from one person to another through coughing, sneezing, touching etc. [4]. Heating at high temperature can kill the bacteria present in food and water. Generally bleaching powder and some disinfectants are used to kill the bacteria present on surface. Antibiotic treatment can help kill or suppress the bacteria.

Escherichia Coli

Escherichia coli are otherwise known as *E. Coli*, gram-negative bacteria with the anaerobic type of respiration. It is a rod shaped, coliform bacteria. The lower intestine of worm blooded organisms generally contains this type of bacteria. A majority of strains of E. coli are serotype but few serotypes can be accounted for carrying serious diseases. Sometimes they are also responsible for the contamination of food. The harmless strains are a part of the microbial that is normally present in the gut. They are responsible for the production of the K2 and also help in preventing the colonization of pathogen bacteria in the intestine, therefore living in a symbiotic relationship [5]. *E. coli* is released into the environment within the facial matter. The bacteria grow rapidly in facial matter under aerobic condition for three days. The gut constitutes of around 0.1% of *E. coli* with other facultative anaerobic [6]. The pathogenic strain of bacteria causes disease through the fecal oral transmission route. As they have the ability to survive to outside the body for some amount of time, there the potential indicator organisms check environmental sample for physical contamination. Research has confirmed that environmentally persistent *E.coli* can survive up to several days outside host and still grow [7]. The bacteria can be grown and cultured easily with less expensive mode in laboratory. They have been widely investigated for over 60 years.

E. coli is chemoheterotroph. So, a source and energy are the most needed component to culture it. *E. coli* is one of the most researched prokaryotic organisms. It is an important species in biotechnology because the majority of work in these fields is concerned with the recombinant DNA [8]. Generally, a large number of strains of E. coli are not harmful for human for they live inside the gut. But some virulent strain can cause stomach flu, tract infections, babe infectious disease, trauma inflammatory bowel disease, and regional enteritis. There are some symptoms and sign embody severe abdominal cramps, diarrhea, trauma inflammatory bowel disease, vomiting, and typically fever. internal organ death (tissue death), hemolytic-uremic syndrome, peritoneal inflammation, mastitis, infection area unit seen in some erratic cases. somehow Severe malady, like lysis pathology syndrome are developed in very early ages; but, in sensible physical form people of all ages are in peril to the unembellished penalties that will arise as a result of being infected with *E. coli* [9].

Salmonella Typhimurium

It is a disease-causing gram-negative bacterium which is mostly found in the lumen of the intestine. Its toxicity is due to the outer membrane which is composed largely of lipopolysaccharides (LPS) that protect the bacteria from the outer environmental factors. The LPS is formed from O-Antigen which is a polysaccharide core and lipid-A that helps it to connect with the outer membrane. The two phosphorylated glucosamines that are attached to fatty acid helps in the formation of lipid-A. The toxicity of this pathogen is determined by these phosphate groups. An enzyme which is carried by all animals act specifically on these phosphate group and remove them in order to protect themselves from these pathogen [10]. Gastroenteritis is caused in human and another animal by the pathogen *Salmonella typhimurium*. Symptoms of typhoid fever in humans is resembled in mice by the pathogen *Salmonella typhimurium*. Typhoid vaccine can be invented by studying these bacteria in mice [11]. A century ago, antibiotics

were discovered and have been used widely in human and animal production. It is used in very small doses, which enhances the disease resistance. However, the main concern is to develop antimicrobial resistance (resistance against microbes) and the transmission of antibiotic genes in bacteria. Since January 2017 application of antibiotic to promote growth in livestock industries was banned in the United States [12]. The production of bioactive compound which can replace the antibiotic act as a growth promoter for food producing animals has been increasing remarkably.

Organic acids and their salts have already been used to increase the pH of the intestine in animal food (acidity the intestinal environment) and increase the nutrient digestively. Formic acid, benzoic acid, citric acid and salts of short chain fatty acids are being used for this purpose. The combination of medium sized fatty acid and organic acids have been found to be beneficial to maintain the intestinal health of animal e.g., Lauric acid. Organic acid can penetrate the bacterial cell as they are lipophilic at their uncharged state. The anion and proton from organic acid increase the osmotic pressure and disrupt the biomolecular components, causing bacterial death. The objectives of the project work are to study the anti-microbial activity of some organic acids *in vitro* condition over gram negative bacteria as they are more used for livestock [13]. Some organic acid likes formic acid, benzoic acid, carboxylic acid and some short chain fatty acids are used as acidifiers to modify intestinal environment. Undissociated and uncharged state in organic acid are capable of bypassing bacterial cell membrane due to their lipophilic nature. Considering the life-threatening pathogenic effect of gram-negative bacteria, it has been a continuous endeavour from wide spectrum of scientific community to develop novel antibacterial agent. Till today, most commonly used antibacterial agents are the derivative of quinolones and fluoroquinolones compounds [14-16]. Herein, we have covered the recent scientific development of these bacteria and their potential inhibitors against theselife-threating pathogenic Gram-negative bacteria. This brief review on inhibitors against gram-negative bacteria may open up a door way to design highly specific inhibitors with high efficacy against the pathogenic gram-negative bacteria.

Reviews of Literatures

Roy et al (2020) synthesized porous silica NPs (MSN) loaded with amoxicillin (an antibacterial drug AMO) for bacterial detection and its inhibition [17]. Anirban Ghosh et al (2020) prepared ananti-bacterial biofilm made from small organic molecule inhibit the bacterial growth [18]. V.K Bajpai et al (2008) inhibit the bacterial growth in food packaging by using Essential Oil and Leaf Extracts of Magnolia liliflora Desr [19]. Xinyi Ren et al (2019) prepared Copper metal–organic frameworks loaded on chitosan film for the efficient inhibition of bacteria which is also used for local infection theraphy [20]. Ahmad Almasoud inhibits the *E. coli* bacteria and *Salmonella typhimurium* by using an organic acid by electro spraying method [21]. Eelena Rusu et al (2017) inhibited the Lactic acid bacteria by using an Oganic compound [22]. Lauren Kovanda et al (2019) prepared an organic molecule inhibitior which inhibit the both gram positive and gram negativebacteria [23]. C.E Carpenter et al (2019) studied how external convent ration like Organic acid anions and pH affect the bacterial inhibition in food package [24]. Sara PONCE DE LEON et al studied the inhibition of Pseudomonas sp. and Moraxella sp by using Lactic acid and Citric Acid [25].

Norha Constanza Bolivar et al inhibit the aquaculture bacteria /pathogen by using various organic salts [26]. Gordan Hayward et al synthesized organic acid made from Kerosene and the carrier tri-octyl phosphine oxides were found to be most bio-compatible of the solvents tested, followed by di-isopropyl ether which inhibit the bacteria [27]. Komsan Phongphakdee et.al. (2015) studied the combined solution of mild ethanol concentration and nisin succeeded in growth reduction of E. coli O157:H7 and Salmonella sp. for 15 min by In vitro solution and solid adherence. It is used for disinfectant solution [28]. Shashidhar Nizalapur et al (2017) studied preparation of N-chloroacetylisatins and their anti-biofilm activities of the compounds against *P. aeruginosa* and *E. coli* [29]. Ramadan Hassan et al (2015) tested organic acids, which used as antifungal were variations in the effect of fungal growth. Acetic acid (10%) has the highest inhibitory effect than citric acid, tartaric acid and formic acid [30]. Aicha El Baaboua et al (2018) studied that the four organic acids tested interfered effectively with the Salmonell as each standard from 0.078to0.312 % of acid, but also depending on acid nature [31]. Sara Ponce De Leon et al (1993) studied extent acetic or citric acid inhibits the growth of Pseudomonas sp. and Moraxella sp. and the degree of inhibition according to acid concentration. The undissociated molecule of the acid is known to be the active antimicrobiaP4-l6) and also to be responsible for pH values [32].

CONCLUSION

Gram-negative bacteria are responsible for causing many noxious diseases such as: neurological abnormalities, gastroenteritis, and life-threatening Typhoid fever in human being and many other animals. The toxicity nature of these bacteria is because of their high tendency to survive in the host body due to the presence of protective cell wall which defends from invasion of exogenous toxic agent. Therefore, developing new organic small molecule with ideally disposed functional unit which can easily prevent the growth of bacteria is always demanding. Herein, we have briefly overviewed different strategic development of inhibitors against gram-negative bacteria. This overview on inhibitors against gram-negative bacteria may be highly valuable for designing new potential inhibitors against gram-negative bacteria.

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REFERENCES

1. Fredrickson, J. K.; Zachara, J. M.; Balkwill, D. L.; Kennedy, D.; Li, S. M.; Kostandarithes, H. M.; Daly, M. J.; Romine, M. F.; Brockman, F. J.; *Appl. and Env. Microbiol.*2004, 70, 4230–41.
2. Dudek, N. K.; Sun, C. L.; Burstein, D.;*Curr. Biol*, 2017,27, 3752–3762.
3. Fang, H.; Kang, J.; Zhang, D.; *Micro. Cell. Fact.* 2017, 16, 15-29.
4. *Whitman, W.B.; Coleman, D. C.; Wiebe, W.J.; Proc. The Nati. Acad. of Sci. of The Unitd. States of Am. 1998, 95, 6578–83.*
5. Russell, J. B.; Jarvis, G. N.; *Jour. of Mole. Microbiol. and Biotechnol.*2001, 3, 265–72.
6. Eckburg, P. B.; Bik, E. M.; Bernstein, C. N.; Purdom, E.; Dethlefsen, L.; Sargent, M.; *Jour. of Bacteriol.*2005, 308, 1635–48.
7. Montealegre, M. C.; Roy, S.; Böni, F.; Hossain, M. I.; Navab-Daneshmand, T.; Caduff.*Jour. of Bacteriol.*2018, 84, 1978–1822.
8. *Kubitschek, H. E.; Jour. of Bacteriol.1990, 172, 94-101.*
9. *Lim, J. Y.; Yoon, J.;Hovde, C. J.; Jour. of Microbiol. and Biotechnol. 2010, 20, 5–14.*
10. James, S.; *American. Soc. for Microbiol.*1995, 63, 437-441.
11. Machinsky, T, T, T. G.; Kessler, M.; Ribeiro, A. M. L.; Moraes, L.; MellodaSilva, I. C.; Mayorga, C.; *Appl. Microbial*. 2015, 40, 2350–2355.
12. Cherrington, C.A.; Hinton, M.; Chopra .*J. Appl. Microbiol*,1990, 68, 69–74.
13. Kashket, E.R.; *FEMS Microbiol. Rev*,1987, 46, 233–244.
14. Asperilla, M.O.; Smego, R.A.; Scott, L.K. *Rev. Infect. Dis.*1990,12, 873-889.
15. Diop, M.B.; Dicois-Dauphin, R.; Tine Jacquelin, A. N.; Thonart; .*Biotech. Agro. Soc. Environ.*2007, 11, 275-283.
16. Martinez,R.C.; Francechini, S.A.; Patta, M.C.; Quintana, S.M.; Canadido, J.C.; De Martinis, E.C.; *Appl. Microbial*, 2009, 48, 269-78.
17. Roy, E.; Nagar, A.; Chaudhary, S.; Pal, S.; *Mater. Sci. inc. Nanomater. & Polymers*. 2020, 5, 722–735.
18. Ghosh, A.; Narayansaswamy, J.; Chatterji, D.; Am. Chem. Soc. Omega. 2020, 8, 25-31.
19. Bajpai, V.; Rahman a.; Dung, N.T.; Huh, M.K.; Chang, S.; *Jour. of food. Sci*. 2008, 73, 6-13.
20. Xinyi, R.; Chengyuan, Y.; Liang, Z.; Sihang, L.; Shuo, S.; Rong, W.; Xu, Zhang; Tianli, Y.; Jing, S.; Jianlong, W.; *Royal. Soc. of Chem.*2019, 11, 11830.
21. Alamsoud, A.; *Google. Scho.* 2015, 35, 312-321.
22. Elena, R.; Ionela, S.; Cristina, C.; Bogdan, I.; Coculescu, H. M.; Stefana, P.; Ander Muresan, Tatiana Vassu, Diana Pelinescu .*MDPI Nanomaterial's*, 2017, 12, 67-78.

23. Lauren, K.; Wen, Z.; Xiaohong, W.; Jia, L.; Xixi, W.; Edward, R. A.; Stefan, V.; Xunde, Li.; Yanhong, L.; Royal *Soc. of Chem*, 24, 37-70.
24. Carpenter, C. E.; Broadbent, J. R.; *The Soc. of food. Sci. and Technol.* 2009, 74, 805-818.
25. Sara, P. D. L.; Norio, I.; Haruo, S.; *Braz. Arch. Bio. Tecnol*. 1993, 44, 80-85.
26. Norha, C.B.; Esmeralda, C. L.; Walter, Q. S.; Edemar, R. A.; Felipe, D. N.; *Braz. Arch. Bio. Tecnol* .2018, 61, 1860-69.
27. Gordon, H.; Isaac, L.; *Braz. Arch. Bio. Tecnol* .1989, 67, 988-1012.
28. Komsa, P.; Sunee, N.; *Journal of food science*. 2015, 80, 10-18.
29. Shashid, H. N.; Onder, K.; Eugene, Y.; Mohan. M. B.; Mike, M.; Mark, C.; David, S.; Kumar, N.; *Royal. Soc. of Chem.*2007, 15, 43-57.
30. Hassan, R.; Sherif, E. K.; Sand, M.; *Inter. Jour. of Adv. in Biol.* 2015, 2, 1-12.
31. Aicha, E. B.; Mohamed, E. M.; Abdelhakim, B.; Omar, B.; Ayoub, K.; Rajae, Z.; Jamal, A.; *Inter. Jour. of Microbiol*. 2018, 15, 288-294.
32. Sara, P. D. L.; Norio, I.; Haruo, S.; *Hokkaid. University. Colle. of Schol. and Acad. Papers*. 1993, 44, 80-85.

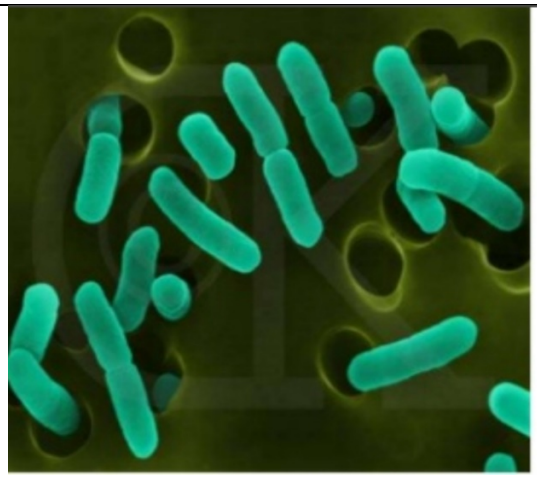

Figure 1: *Escherichia Coli*

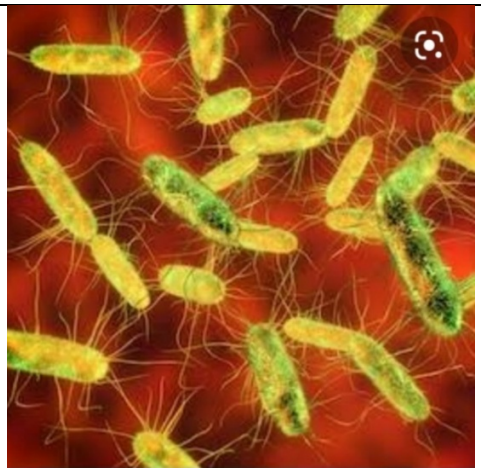

Figure 2: *Salmonella typhimurium*

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SHORT COMMUNICATION ARTICLE

Understanding the Significance of Protecting Wood Fossils to Preserve Palaeobotanical Past in Shahebgunj District, Jharkhand

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ABSTRACT

A wood fossil park in Mandro of Shahebgunj district in Jharkhand, India is at the risk of rapid weathering and decay. Sedimentological strata of stratigraphic columns frequently host wood fossils housing vital information about the past climate and ecology besides hinting at the possible evolutionary track. An understanding of wood fossils through palaeobotany reveals detailed track of development to the present fauna. Therefore, it is essential to conserve and study the exposed fossils.

Keywords: Plant fossil, paleobotany, fossil preservation and conservation, evolution, importance of fossil.

Rajmahal hills of Mandro in Shahebgunj district, Jharkhand is a storehouse of plant fossils that were previously thought to beabout 70 to 110 million years old. Traps and intertrappean beds are distinguishing features of the Rajmahal hills [1] (Fig. 1). Latest Ar/Ar date of the youngest lava flow of Rajmahal Formation has been found to be 118 Ma[2,3]. An understanding of the species distribution through space requires knowledge regarding its distribution through time. Distributions are constantly modifying due to shifts in climatological conditions or ecological factors, aggravated by anthropogenic activities. The history of a species reveals vital information about its range expansion or contraction. Such information leads to the understanding of factors responsible for limiting the species distribution. These intertrappean sediments spread out all over the Rajmahal Basin, except in its western part[4–7]. The basin has been extensively researched in the past by notable geologists due to the presence of rich mega plant *Ptilophyllum* flora in the Intertrappean beds along with ferns, conifers and cycads[8–11]. *Ptilophyllum* flora is one of the three major plant species that dominated the Indian subcontinent between Early Permian and Early cretaceous periods with *Ptilophyllum* being the index fossil of Early Cretaceous Era[12]. Global fossil records indicate that early cretaceous era marked the base for plant revolution that carved way for the evolution of modern existing flora. This period is regarded as the "Dawn of New Era" or the "Era of Flowering Plants"[13]. This marks the Upper Gondwana period in Rajmahal hills. A major shift in plant species type is observed between the Lower and Upper Gondwana floral communities i.e., the appearance of Gymnosperms is witnessed in the Upper Gondwana as an

indicator. Spectroscopic studies reveal the paleo environmental conditions of the plant fossils[14]. The Rajmahal Basin houses one of the most-developed sequences of the Upper Gondwana sediments which dates back to the Lower Cretaceous period. Stratigraphic sequence was first developed by V. Ball in 1877 followed by S. Sengupta in 1988 and Tripathi et al. in 2008[15–18] (Table 1). The uniqueness of this sequence lies in the fact that a volcanic lava flow coexists with sedimentary beds. Mandro hosts a majority of these plant fossils because of which a fossil park has been set up in the region which is a great step for preservation of geo-heritage sites.The basin is about 600 meters thick and spreads over 4100 sq. km, comprisinga series of volcanic lava flows (Rajmahal Traps) along with intertrappean sedimentary beds with basalt being the dominant rock.

The first record of the plant fossil dating back to the Jurassic era was cited in the year 1931 [7,19,20]. Mining has always been a problem since it not only destroys the present flora but also the petrified flora. It is extremely commendable that to counter the same in 2008, the state government of Jharkhand had signed a memorandum of understanding with the Birbal Sahni Institute of Palaeosciences (BSIP), Lucknow, and National Building Construction Corporation, Ranchi for setting up the fossil park. Post this great initiative the site has also been included as a geo-tourism site[21]. Proper entrance and a park with features important for sustaining a Fossil park is present and well maintained. But the surrounding areas have exposed fossils where absence of a proper demarcated boundary or a proper entrance in nearby areas of the park allows a free trespass for passers. Sections of the exposed fossils are demolished and often carried away by tourists and locals as souvenirs thereby depleting the fossil extent (Figure 2b & 2b). Most of the natives are unaware of the importance of the area and in most cases cause damage unintentionally. It is confirmed after conducting a local survey consisting of 50 individuals of the vicinity revealing that local jewellery shops sell these fossilized plant species as ornaments to tourists at high prices. Fossils play a major role in reconstructing the past. It not only gives us an insight to prehistoric life forms but also the environment and evolutionary changes that occurred in the course of time. From the geological point of view fossils help in dating rocks. Most importantly fossilisation is a rare process which should be preserved and studied to understand the past and take necessary steps for a sustainable future. *Ptilophyllum* flora, like other plant fossils are especially useful as they give a cognizance to the past climate and vegetation on a broader aspect. Plant fossils from the earliest times provide information about the surrounding with an indication of the paleoclimatic conditions. The present-day plants indicate the present environment and can be significantly correlated to the past climate revealing necessary information. It also serves as an essential tool for enquiry of fossil fuels like coal and oil.

As a management practice, urgent actions need to be adopted by concerned authorities for the restoration of the nearby areas of the park. A lot of effort has been directed through several news correspondents to communicate the importance of these plant fossils to the concerned authorities. BSIP, Lucknow has taken up much effort in constructing the major area but some adjoining village areas still need proper maintenance and protection. Such efforts have resulted in a plan of management that has been duly proposed by the concerned authorities. Yet it is important that further steps are taken. Capacity building programmes might be organized in the future for generating proper preservation awareness among the local people. Unique identification markers might be generated and catalogued for management purposes. Areas that are exposed in the adjoining village areas can be protected separately to prevent damage caused by sunlight and rain. Therefore, it is significant to understand wood fossils bridgingthe gap between the past, the present and future.

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during the tenure the first author was a student at University of Calcutta as part of an individual work. The currently affiliated institute has no link to this work. This short communication is dedicated to Dr.Ashis Kumar Das.

REFERENCES

1 Kent, W., Saunders, A. D., Kempton, P. D., and Ghose, N. C., Rajmahal basalts, eastern India: Mantle sources and melt distribution at a volcanic rifted margin. *Geophys. Monogr. Ser.*, 1997, **100**, 145–182.
2 Agrawal and Rama, Chronology of Mesozoic volcanics of India. *Proc. Indian Acad. Sci.*, 1976, **84 A**, 157–179.
3 McDougall, I. and McElhinny, M. W., The Rajmahal Traps of India - KAr ages and palaeomagnetism. *Earth Planet. Sci. Lett.*, 1970, **9**, 371–378.
4 Sinha, A. and Rais, S., Granulometric Analysis of Rajmahal Inter-Trappen Sedimentary Rocks (Early Cretaceous), Eastern India, Implications for Depositional History. *Int. J. Geosci.*, 2019, **10**, 238–253.
5 Singh, A. P., Kumar, N., and Singh, B., Magmatic underplating beneath the Rajmahal Traps: Gravity signature and derived 3-D configuration. *Proc. Indian Acad. Sci. Earth Planet. Sci.*, 2004, 113, 759–769.
6 Kale, V. S., Cretaceous volcanism in peninsular india: rajmahal - sylhet & deccan traps. In *Springer Geology* 2020, pp. 1–61.
7 Kent, R. W., Pringle, M. S., Müller, R. D., Saunders, A. D., and Ghose, N. C., 40Ar/39Ar geochronology of the Rajmahal Basalts, India, and their relationship to the Kerguelen plateau. *J. Petrol.*, 2002, 43, 1141–1153.
8 Banerji, J., Megafloral diversity of the upper Gondwana sequence of the Rajmahal Basin, India. *J. African Earth Sci.*, 2000, 31, 133–144.
9 Archana Tripathi and Atanu Ray, Palynostratigraphy of the Dubrajpur Formation (Early Triassic to Early Cretaceous) of the Rajmahal Basin, India. *Am. Assoc. Stratigr. Palynol.*, 2006, 30, 133–149.
10 Tripathi, A., Jana, B. N., Verma, O., Singh, R. K., and Singh, A. K., Early cretaceous palynomorphs, dinoflagellates and plant megafossils from the Rajmahal Basin, Jharkhand, India. *J. Palaeontol. Soc. India*, 2013, 58, 125–134.
11 Murthy, S., Mendhe, V. A., Uhl, D., Mathews, R. P., Mishra, V. K., and Gautam, S., Palaeobotanical and biomarker evidence for Early Permian (Artinskian) wildfire in the Rajmahal Basin, India. *J. Palaeogeogr.*, 2021, 10, 21.
12 Rajanikanth, A., Geological age of the Ptilophyllum flora. *Mem. Geol. Soc. India*, 2000, 46, 245–256.
13 A. C. Seward, *FOSSIL PLANTS* 1931.
14 Da Silva, J. H., Freire, P. T. C., Abagaro, B. T. O., et al., Spectroscopic studies of wood fossils from the Crato Formation, Cretaceous Period. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, 2013, 115, 324–329.
15 Ghose, N. C., Singh, S. P., Singh, R. N., and Mukherjee, D., Flow stratigraphy of selected sections of the Rajmahal basalts, eastern India. *J. Southeast Asian Earth Sci.*, 1996, 13, 83–93.
16 Sengupta, S., Upper Gondwana stratigraphy and palaeobotany of Rajmahal Hills, Bihar, India. *Geol. Surv. India Palaeontol. Indica*, 1988, 48, 1–180.
17 Ball, V., Geology of the Rajmahal Hills. *Mem. Geol. Surv. India*, 1877, **13**, 155–248.
18 Tripathy, A., Palynochronology of Lower Cretaceous Volcanosedimentary succession of the Rajmahal Formation in the Rajmahal Basin. *Cretac. Res.*, 2008, pp. 913–924.
19 Rao, M. R. D. S., the Jurassic Flora of the Rajmahal Hills. *Birbal Sahni Inst. Palaeobot. Lucknow.-*, 1972, 3–14.
20 Ajaykumar, B., Joseph, S., Abhilash, P. C., Mohan, M., Anitha, K., and Thomas, A. P., Fossil wood: A key to the past. *Wood Types, Prop. Uses*, 2011, 169–180.
21 Wadhawan S.K. (2021) Geoheritage and Potential Geotourism in Geoparks—Indian Perspective. In: Singh R., Wei D., Anand S. (eds) Global Geographical Heritage, Geoparks and Geotourism. Advances in Geographical and Environmental Sciences. Springer, Singapore.

Table 1: Geological Succession exposed at, The Rajmahal area, eastern Jharkhand (after Ball, 1877; Sengupta 1988; Tiwari &Tripathi 1995; Ghose*et al*., 1996)

Group	Formation	Lithology	Age
		Alluvium	Recent
		Laterite	
Upper Gondwana	Rajmahal	Basalt and acid volcanics with intertrappean beds of pyroclastic material, argillaceous, and arenaceous sediments often contain plant fossils (*Ptilophyllum* flora) and bentonite deposits in the lower sequence Igneous contact	Lower Cretaceous
	Dubrajpur	Pebbly ferruginous sandstone, conglomerate Triassic to and grit passing into siltstone and shale Usually forms high ridges and scarps Disconformity	Early Cretaceous
	Barakar	Sandstone, shale, and carbonaceous shale with coal seam Lower	Lower Permian Unconformity
	Talchir	Boulder bed, fine-grained sandstone, Yellow or green shale, highly weathered	Lower Corboniferous Unconformity
	Proterozoic	Chhotanagpur gneiss-granulite complex	

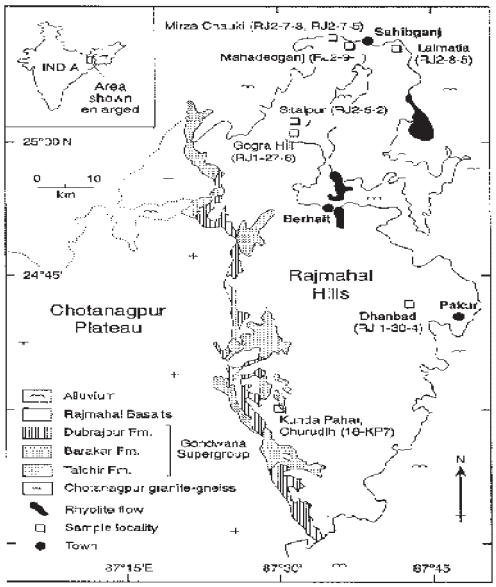
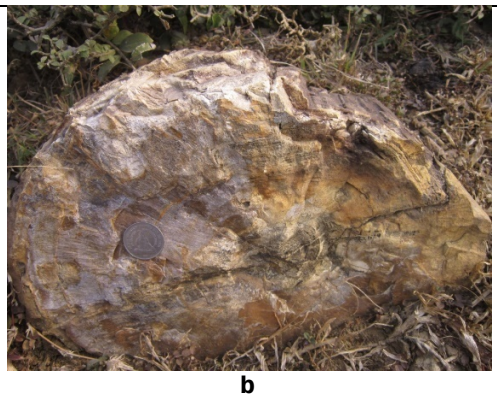


Fig 1: Location map of the Rajmahal Hills, Jharkhand state, eastern India (Kent et al. 1997)

a



b

Fig 2 (a & b): Unpreserved exposure of fossilized plant remains.

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RESEARCH ARTICLE

Impact of Non-Cognitive Factors on Academic Performance in Higher Education

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ABSTRACT

Non-cognitive factors determine academic outcomes to a large extent hence the role of non-cognitive factors in academic performance is an important area of research. The current study aimed to investigate the effects of self-efficacy beliefs, self-regulation, perseverance, motivation on academic performance. Cross-sectional survey was used to collect data from 438 university students. The students filled out four questionnaires: Grit-S. college academic self-efficacy scale (CASES), intrinsic value subscale of Motivated Strategies for Learning Questionnaire (MSLQ) and Brief Self Control Scale ((BSCS). Academic performance was measured using the GPA obtained in the past year. Structural equation modelling was used to test the model. Results confirmed a significant impact of academic self-efficacy on academic motivation and self-regulation which in turn effected the academic performance of students. Also, the effect of grit on academic motivation was confirmed.

Keywords: Non-Cognitive factors, Academic Performance, Higher Education.

INTRODUCTION

Academic performance in a true sense is interplay between the cognitive and non-cognitive factors. However, common people's lexicon remains oblivious to the non-cognitive factors even though many studies have highlighted their importance. Researches in the area of educational psychology focus on improving academic outcomes for learners, and cognitive skills like academic skills and content knowledge [1] have been considered very crucial for

student achievement [2]. However it has been shown that students' performance cannot be predicted by academic factors alone [3]. Maximizing academic outcomes has emerged as a potential area of research in recent years. This new interest has made the researchers integrate non-cognitive skills and factors into the framework of education. Non-cognitive skills may be understood as attitudinal beliefs, behaviours, and strategies which help to succeed in school and workplace. Some of the widely researched non-cognitive skills are motivation, perseverance, and self-control. Recent researches in the areas of economics, education and social sciences have emphasized the role of non-cognitive skills in educational achievement [4-5]; [6] and employability [7]. Researchers also suggest the importance of developing these factors to maximize the returns in areas of education and employment [8] Growing evidence is available for the role of factors like resilience, self-management, motivation, self-regulation, self-efficacy and social skills [9-10] in academic outcomes.

Literature on non-cognitive factors uses many terms like 'skills', 'traits', 'soft-skills', 'character traits', 'life-skills' and 'behaviour' to denote the non-cognitive factors. These factors are termed 'non-cognitive' to mark their distinction from the cognitive and academic skills as assessed by tests or teacher assessments. However, the distinction between 'cognitive' and 'non-cognitive' is conceptually misleading as it tries to denote a sense of flawed differentiation between the two. As put by [11] "few aspects of human behaviour are devoid of cognition" [12]. A review of related literature reveals a variety of factors being considered as non-cognitive components contributing to academic success. According to [13] college readiness comprises eight non-cognitive components, namely positive self-concept, realistic self-appraisal, understanding and dealing with racism, preference for long term goals, having an available support person, successful leadership experience, community and knowledge acquired nontraditionally. Non cognitive factors lead one to engage in academic behaviours which are associated with academic performance [14] and academic behaviours like student engagement at school are crucial when it comes to academic success [15] as they involve behavioral, cognitive as well as emotional engagement. Non cognitive factors like time management, self-regulation and motivation are important for later life outcomes and were observed to be related to academic performance [16] In another study non cognitive factors were found to be correlated with GPA [17-18] conducted a meta-analysis of over 200 studies which aimed at studying the social emotional learning (SEL) based intervention plans for school children (ages 5-18). The results of the meta analysis revealed that SEL interventions resulted in improved test score and grades. Therefore, a discourse about academic performance is incomplete without the mention of non-cognitive factors.

There is no consensus on the list of non-cognitive factors. Based on the taxonomy of such factors, five general categories have been identified: learning strategies, academic mindsets, perseverance, academic behaviours and social skills [19-20].Another approach groups these non-cognitive factors and psychosocial attitudes into two categories. The first category is the learning strategies, which include several overlapping constructs like metacognition, self-regulation, goal setting, academic performance skills such as problem solving. Learning strategies broadly refers to students' ability to set goals and adopt and adapt strategies to reach their goals. The second category is of mindsets described in the literature on non-cognitive factors. This includes the beliefs about the self, competencies and the process of learning. The distinction between growth and fixed mindsets[21-22] and their impact on effort and academic achievement is of special significance in this context.

Theoretical model and hypothesis development

Based on the existing research it can be concluded that non-cognitive variables are associated with the academic achievements of students. Factors such as academic self-efficacy, motivation and self-regulation affect academic outcomes positively [23-24][25]. The present research aims to find the impact of academic self-efficacy, grit, academic motivation and academic self-esteem on academic performance of students and also to study the intercorrelations among these factors (Figure 1). Self efficacy is an important predictor of achievement. Based on Bandura's [26] concept of general self-efficacy, academic self-efficacy is the students' belief in their ability to master an academic subject or their confidence in their ability to successfully perform an academic task. Self efficacy has been related to

academic performance, this relationship was mediated by students' expectancy value beliefs. [27] Therefore, it can be stated that:

H1: Self-efficacy significantly effects the self-regulation of students
H2: Self-efficacy significantly effects the academic motivation of students
Grit is the passion and perseverance for long term goals that enables students to consistently be interested and put effort in the same goal for a long period of time. People who are high on grit are able to persist even in adversities and are not demotivated by them. [28-29] observed that grit was positively related to academic achievement in math and attitudes towards math and science was positively related to academic achievement in both math and science. Hence, the following hypotheses can be formulated

H3: Grit significantly effects the self-regulation of students
H4: Grit significantly effects the academic motivation of students
Academic motivation is rooted in the need for achievement and entails a variety of concepts like task values, goals, achievement motives which makeup the factors behind behaviors that contribute to academic functioning. According to the hierarchical model of achievement motivation need for achievement is one of the factors which is responsible for predisposing individuals to choose particular types of achievement goals. Academic motivation predisposes an individual to adopt learning goals which have been positively associated with academic outcomes [30] and higher grades [31]. Hence, the following hypotheses can be formulated:

H5: Academic motivation has a significant influence on academic performance of students
The self-regulation of learning is an active process where individuals deliberately direct their thoughts and actions towards their goals. According to [32] academic self-regulation is the extent to which students show conscientiousness in their approach towards academic goals. Zimmerman [33] posited that students who are able to self-regulate their learning are able to learn better and this occurs in a four step cycle which involves self evaluation, goal setting, implementation and evaluation. A study by Sahranavard, Miri and Salehiniya [34] revealed that self-regulation was related to educational performance. Low levels of behavioral self-regulation predict poor academic achievement and peer rejection [35] Thus, it is hypothesized that:

H6: Self-regulation has significant impact on academic performance of students

MATERIALS AND METHODS

Sample description
This cross sectional study involved 438 students enrolled in various university programmes at UG and PG levels. The sample is drawn from 35 private universities across Rajasthan. The age of respondents is 18-26 years (average age 20.4); balanced distribution by gender (44.8% of women, 55.2% of men). The sample comprised students from UG (63%) and PG (37%) courses, technical (41.6%) and non-technical (58.4%) courses.

Tools
The survey method was used to test the theoretical model. Standardized tools were used to collect data. All tools, in accordance with the objectives of the current research have been briefly described:

Grit was measured using the short grit scale (Grit-S) [36]
It is an 8 item scale to measure consistency of interest (e.g., "I have been obsessed with a certain idea or project for a short time but later lost interest"), and perseverance of effort (e.g., "I finish whatever I begin"). Grit-S is widely used in social science research to assess the latent construct grit. The response options ranged from 1 (not at all like me) to

6 (very much like me). Responses to items on the interest subscale were reversed coded. Higher scores indicate higher levels of grit.

Academic self-efficacy was measured using the College Academic Self Efficacy Scale (CASES) [37].
The scale has 33 items to measure self-efficacy in 33 academic behaviors. Responses are obtained on a five-point Likert-type scale. Each item measures degrees of confidence ranging from quite a lot (5 points) to very little (1 point). Higher scores indicate higher college academic self-efficacy.

Academic motivation was measured using the intrinsic value subscale of Motivated Strategies for Learning Questionnaire (MSLQ). [38]. MSLQ has 44 items to investigate students' perceptions on learning and the strategies employed by them. The scale is widely used and is divided into six subscales. The individual scales of MSLQ can be used independently. The intrinsic value subscale has 9 items to be answered on a five point Likert scale.

Academic Self-regulation was measured using the Brief Self Control Scale (BSCS; **[39].**
It is a five point Likertscale consisting of 13 items to assess dispositional self-regulation. The BSCS focuses on processes involving self-control (e.g., breaking a habit, working toward long-term goals).

Academic Performance
This was measured using the GPA in the last exam.

Data Analytic Procedure
SPSS 22.0 and Amos 21.0 were used to manage and analyze the data. Structural equation modeling was used to test the proposed hypotheses. Goodness of fit of the proposed model was tested using the Root Mean Square Error of Approximation (RMSEA), the Tucker-Lewis Index (TLI), and the Comparative Fit Index (CFI; [40]; [41]

RESULTS AND DISCUSSION

SPSS 22 was used to analyze the collected data. Table 1 shows the descriptive statistics, correlation among the study variables and Cronbach's alpha values obtained in the study sample. The correlation matrix shows a positive correlation among all the variables. Academic performance is also positively related to academic motivation, academic self-efficacy, grit and academic self-regulation.

Measurement Model
Before proceeding with the path analyses model goodness of fit was evaluated for the measurement model. CFA was done for all the scales used in the study and NFI, CFI, TLI, IFI, RMSEA and CMIN/DF were used to examine the model fit to the data. Table 2 shows the results of adequacy of the research constructs.

Structural Model Assessment (SEM)
SEM was used to test the model fit of the proposed model and to test the hypotheses. The output is shown in table 3. Figure 2 shows the estimated paths from the analysis. H1, H2, H3, H5, and H6 were supported and H4 was not supported (Table 3). The hypotheses were tested usingstandardized path coefficients (β) and critical values (CR). Further, table 4 below shows the output summary for hypotheses tests at different significance levels (.05, .01, and .001).

DISCUSSION

The current study supported academic motivation, academic self-efficacy, grit, academic self-regulation as predictors of academic performance. Results confirmed the influence of academic self-efficacy on academic self-regulation and

academic self-esteem which is in accordance with previous researches as correlational relationship was found between self-efficacy, self-regulation of learning and academic achievement [42] Beliefs about doing well in the task, i.e. expectancy beliefs influence the choices made by individuals, the fervor of their efforts [43] and the tendency to choose appropriate academic behaviours over others, i.e. regulating their behaviour. In the light of the obtained results, self-efficacy beliefs can be understood as a source of motivation and directed behaviour. In a similar vein, the structural model tested by Bong et al. [44] revealed that self-beliefs (self-efficacy and self-concept) are good predictors of task value. Motivational theories also posit that how individuals perceive their competency motivates the individual towards initiating action and also directing and channelizing energies in the right direction and also[45-46] If learners believe in their efficacy they will feel more competent and also invest more efforts to perform better.

Another finding of this study was the influence of grit on academic motivation. Grit as a personality trait is a tendency to maintain passion and effort towards long-term goals [47- 48] Individuals with higher grit show perseverance and consistency and are known to be higher motivated. They adopt strategic behaviour to achieve their goals and show sustained efforts [49] Grit is associated with long term persistence and motivation. However, the impact of grit on self-regulation (H4) was not supported. This finding lends support to other findings which demonstrate the two dimensions of grit: perseverance of effort and consistency of interest differ in the way they are related to self-regulatory mechanisms [50].

The study results also confirmed the effect of academic motivation and academic self-regulation on academic performance. These findings support the existing literature on motivation [51] in traditional classrooms and online learning environments. [51] High levels of intrinsic motivation have been associated with higher performance by many researchers [52-53]. According to the value expectancy model, the students' self-reported task value plays an important role in working towards the task. An intrinsic motivation in the task is a strong predictor of performance. Consistent with social cognitive models of learning [54]; [55] findings support that performance can be partly explained through motivational beliefs. Students high on self-regulation also perform better as they are better able to plan, monitor and control their classroom academic tasks and avoid distraction. It is not necessary that students enjoy the task at hand, but if one has to perform well, it becomes important to use strategies to regulate one's behaviour. The influence of academic self-regulation on academic performance is in line with previous researches where a significant correlation was found between self-regulation and educational performance [56] and self-regulation was observed to be a significant predictor of student grades [57].

CONCLUSIONS

The results provided empirical support to the role of non-cognitive factors in predicting academic performance. Intrinsic motivation and self-regulatory behavior turned out to be significant predictors of academic performance. However, the results obtained did not provide support to the proposed effect of grit on academic self-regulation probably because the study considered grit as a composite measure.

REFERENCES

1. Finn, Robert D., Alex Bateman, Jody Clements, Penelope Coggill, Ruth Y. Eberhardt, Sean R. Eddy, Andreas Heger et al. "Pfam: the protein families database." *Nucleic acids research* 42, no. D1 (2014): D222-D230.
2. Farkas, George. "Cognitive skills and noncognitive traits and behaviors in stratification processes." *Annual review of sociology* 29, no. 1 (2003): 541-562.
3. Astin, Alexander W., and Helen S. Astin. "Undergraduate science education: the impact of different college environments on the educational pipeline in the sciences. Final report." (1992).

4. Bowles, Samuel, and Herbert Gintis. "The inheritance of inequality." *Journal of economic Perspectives* 16, no. 3 (2002): 3-30.
5. Gracia-Iguacel, Carolina, Emilio González-Parra, Guillermina Barril-Cuadrado, Rosa Sánchez, Jesús Egido, Alberto Ortiz-Arduán, and Juan J. Carrero. "Definiendo el síndrome de desgaste proteico energético en la enfermedad renal crónica: prevalencia e implicaciones clínicas." *Nefrología (Madrid)* 34, no. 4 (2014): 507-519.
6. Stankov, Lazar, Suzanne Morony, and Yim Ping Lee. "Confidence: the best non-cognitive predictor of academic achievement?." *Educational psychology* 34, no. 1 (2014): 9-28.
7. Gutman, Leslie Morrison, and Ingrid Schoon. "The impact of non-cognitive skills on outcomes for young people." *Education Endowment Foundation*
8. Heckman, James J., Jora Stixrud, and Sergio Urzua. "The effects of cognitive and noncognitive abilities on labor market outcomes and social behavior." *Journal of Labor 9economics* 24, no. 3 (2006): 411-482.
9. Durlak, Joseph A., Roger P. Weissberg, Allison B. Dymnicki, Rebecca D. Taylor, and Kriston B. Schellinger. "The impact of enhancing students' social and emotional learning: A meta-analysis of school-based universal interventions." *Child development* 82, no. 1 (2011): 405-432.
10. Jones, Stephanie M., and Suzanne M. Bouffard. "Social and emotional learning in schools: From programs to strategies and commentaries." *Social policy report* 26, no. 4 (2012): 1-33.
11. Borghans, Lex, Angela Lee Duckworth, James J. Heckman, and Bas Ter Weel. "The economics and psychology of personality traits." *Journal of human Resources* 43, no. 4 (2008): 972-1059.
12. Borghans, Lex, Huub Meijers, and Bas Ter Weel. "The role of noncognitive skills in explaining cognitive test scores." *Economic inquiry* 46, no. 1 (2008): 2-12.
13. Sedlacek, H., J. O. R. G. Czech, R. A. M. A. C. H. A. N. D. R. A. Naik, G. U. R. M. E. E. T. Kaur, P. E. T. E. R. Worland, M. I. C. H. A. E. L. Losiewicz, B. E. R. N. A. R. D. Parker et al. "Flavopiridol (L86 8275; NSC 649890), a new kinase inhibitor for tumor therapy." *International journal of oncology* 9, no. 6 (1996): 1143-1168.
14. Wanzer, Dana, Elyse Postlewaite, and Nazanin Zargarpour. "Relationships among noncognitive factors and academic performance: Testing the University of Chicago Consortium on School Research model." *AERA Open* 5, no. 4 (2019): 2332858419897275.
15. Reschly, Amy L., and Sandra L. Christenson. "Jingle, jangle, and conceptual haziness: Evolution and future directions of the engagement construct." In *Handbook of research on student engagement*, pp. 3-19. Springer, Boston, MA, 2012.
16. Duckworth, Angela L., and Martin EP Seligman. "Self-discipline outdoes IQ in predicting academic performance of adolescents." *Psychological science* 16, no. 12 (2005): 939-944.
17. Erten, İsmail Hakkı, and Robert L. Burden. "The relationship between academic self-concept, attributions, and L2 achievement." *System* 42 (2014): 391-401.
18. Durlak, Joseph A., Roger P. Weissberg, Allison B. Dymnicki, Rebecca D. Taylor, and Kriston B. Schellinger. "The impact of enhancing students' social and emotional learning: A meta-analysis of school-based universal interventions." *Child development* 82, no. 1 (2011): 405-432.
19. [19] Farrington, Camille A., Melissa Roderick, Elaine Allensworth, Jenny Nagaoka, Tasha Seneca Keyes, David W. Johnson, and Nicole O. Beechum. *Teaching Adolescents to Become Learners: The Role of Noncognitive Factors in Shaping School Performance--A Critical Literature Review*. Consortium on Chicago School Research. 1313 East 60th Street, Chicago, IL 60637, 2012.
20. Atkins-Burnett, Sally, Camila Fernández, Lauren Akers, Jessica Jacobson, and Claire Smither-Wulsin. *Landscape analysis of non-cognitive measures*. Mathematica Policy Research, 2012.
21. Dweck, Carol S. "Mind-sets." *Principal leadership* 10, no. 5 (2010): 26-29.
22. Lerner, Richard M., Kathleen Leonard, Kristen Fay, and Sonia S. Issac. "Continuity and discontinuity in development across the life span: A developmental systems perspective." *Handbook of life-span development* (2011): 141-160.
23. Stankov, Lazar. "Unforgiving Confucian culture: A breeding ground for high academic achievement, test anxiety and self-doubt?." *Learning and Individual Differences* 20, no. 6 (2010): 555-563.

24. Gracia, José López, Alejandro Casino Martínez, and Cristina Aybar Arias. "Efectos financieros y estratégicos sobre la estructura de cápital de la pequeña y median empresa." *Moneda y crédito* 219 (2004): 71-100.
25. Davidson, Peter Alan. *Turbulence: an introduction for scientists and engineers*. Oxford university press, 2015.
26. Bandura, Albert. "Self-efficacy." *The Corsini encyclopedia of psychology* (2010): 1-3.
27. Doménech-Betoret, Fernando, Laura Abellán-Roselló, and Amparo Gómez-Artiga. "Self-efficacy, satisfaction, and academic achievement: the mediator role of Students' expectancy-value beliefs." *Frontiers in psychology* 8 (2017): 1193.
28. Duckworth, Angela L., Christopher Peterson, Michael D. Matthews, and Dennis R. Kelly. "Grit: perseverance and passion for long-term goals." *Journal of personality and social psychology* 92, no. 6 (2007): 1087.
29. Al-Mutawah, Masooma Ali, and Moosa Jaafar Fateel. "Students' Achievement in Math and Science: How Grit and Attitudes Influence?." *International Education Studies* 11, no. 2 (2018): 97-105.
30. Eppler, Marion A., and Beverly L. Harju. "Achievement motivation goals in relation to academic performance in traditional and nontraditional college students." *Research in Higher education* 38, no. 5 (1997): 557-573.
31. Steinmayr, Ricarda, Anne F. Weidinger, Malte Schwinger, and Birgit Spinath. "The importance of students' motivation for their academic achievement–replicating and extending previous findings." *Frontiers in psychology* 10 (2019): 1730.
32. Allen, Jeff, Steven B. Robbins, Alex Casillas, and In-Sue Oh. "Third-year college retention and transfer: Effects of academic performance, motivation, and social connectedness." *Research in Higher Education* 49, no. 7 (2008): 647-664.
33. Zimmerman, Barry J. "Academic studing and the development of personal skill: A self-regulatory perspective." *Educational psychologist* 33, no. 2-3 (1998): 73-86.
34. Sahranavard, Sara, Mohamad Reza Miri, and Hamid Salehiniya. "The relationship between self-regulation and educational performance in students." *Journal of education and health promotion* 7 (2018).
35. McClelland, Megan M., Frederick J. Morrison, and Deborah L. Holmes. "Children at risk for early academic problems: The role of learning-related social skills." *Early childhood research quarterly* 15, no. 3 (2000): 307-329.
36. Duckworth, Angela Lee, and Patrick D. Quinn. "Development and validation of the Short Grit Scale (GRIT–S)." *Journal of personality assessment* 91, no. 2 (2009): 166-174.
37. Owen, Steven V., and Robin D. Froman. "Development of a College Academic Self-Efficacy Scale." (1988).
38. Pintrich, Paul R., and Elisabeth V. De Groot. "Motivational and self-regulated learning components of classroom academic performance." *Journal of educational psychology* 82, no. 1 (1990): 33.
39. Tangney, June Price, Angela L. Boone, and Ronda Dearing. "Forgiving the self: Conceptual issues and empirical findings." In *Handbook of forgiveness*, pp. 167-182. Routledge, 2007.
40. Yu, Ching-Yun. *Evaluating cutoff criteria of model fit indices for latent variable models with binary and continuous outcomes*. Vol. 30. Los Angeles, CA: University of California, Los Angeles, 2002.
41. Marsh, Herbert W., Kit-Tai Hau, and Zhonglin Wen. "In search of golden rules: Comment on hypothesis-testing approaches to setting cutoff values for fit indexes and dangers in overgeneralizing Hu and Bentler's (1999) findings." *Structural equation modeling* 11, no. 3 (2004): 320-341.
42. Agustiani, Hendriati, Surya Cahyad, and Muwaga Musa. "Self-efficacy and self-regulated learning as predictors of students academic performance." *The Open Psychology Journal* 9, no. 1 (2016).
43. Wigfield, Allan, and Jacquelynne S. Eccles. "The development of achievement task values: A theoretical analysis." *Developmental review* 12, no. 3 (1992): 265-310.
44. Bong, Mimi, Catherine Cho, Hyun Seon Ahn, and Hye Jin Kim. "Comparison of self-beliefs for predicting student motivation and achievement." *The Journal of Educational Research* 105, no. 5 (2012): 336-352.
45. Schunk, Dale H. "Self-efficacy and academic motivation." *Educational psychologist* 26, no. 3-4 (1991): 207-231.
46. Deci, Edward L., Robert J. Vallerand, Luc G. Pelletier, and Richard M. Ryan. "Motivation and education: The self-determination perspective." *Educational psychologist* 26, no. 3-4 (1991): 325-346.
47. Duckworth, Angela L., Christopher Peterson, Michael D. Matthews, and Dennis R. Kelly. "Grit: perseverance and passion for long-term goals." *Journal of personality and social psychology* 92, no. 6 (2007): 1087.

48. Duckworth, Angela Lee, and Patrick D. Quinn. "Development and validation of the Short Grit Scale (GRIT–S)." *Journal of personality assessment* 91, no. 2 (2009): 166-174.
49. Eskreis-Winkler, Sarah, Giuseppe Corrias, Serena Monti, Junting Zheng, Marinela Capanu, Simone Krebs, Maggie Fung, Scott Reeder, and Lorenzo Mannelli. "IDEAL-IQ in an oncologic population: meeting the challenge of concomitant liver fat and liver iron." *Cancer imaging* 18, no. 1 (2018): 1-7.
50. Hagger, Martin S., and Kyra Hamilton. "Motivational predictors of students' participation in out-of-school learning activities and academic attainment in science: An application of the trans-contextual model using Bayesian path analysis." *Learning and Individual Differences* 67 (2018): 232-244.
51. Schunk, Dale H., and Barry J. Zimmerman, eds. *Self-regulated learning: From teaching to self-reflective practice*. Guilford Press, 1998.
52. Robbins, Steven B., Kristy Lauver, Huy Le, Daniel Davis, Ronelle Langley, and Aaron Carlstrom. "Do psychosocial and study skill factors predict college outcomes? A meta-analysis." *Psychological bulletin* 130, no. 2 (2004): 261.
53. Pintrich, Paul R., and Elisabeth V. De Groot. "Motivational and self-regulated learning components of classroom academic performance." *Journal of educational psychology* 82, no. 1 (1990): 33.
54. Pintrich, Paul R. "The role of motivation in promoting and sustaining self-regulated learning." *International journal of educational research* 31, no. 6 (1999): 459-470.
55. Zimmerman, Barry J., and Dale H. Schunk, eds. *Self-regulated learning and academic achievement: Theoretical perspectives*. Routledge, 2001.
56. Sahranavard, Sara, Mohamad Reza Miri, and Hamid Salehiniya. "The relationship between self-regulation and educational performance in students." *Journal of education and health promotion* 7 (2018).
57. Nota, Laura, Salvatore Soresi, and Barry J. Zimmerman. "Self-regulation and academic achievement and resilience: A longitudinal study." *International journal of educational research* 41, no. 3 (2004): 198-215.

Table 1. Descriptive Statistics

	Mean	SD	Academic Motivation	Academic Self Efficacy	Grit	Academic Self-Regulation	Academic Performance
Academic Motivation	25.5	4.46	-	.785**	.440**	.564**	.725**
Academic Self Efficacy	83.99	22.24		-	.724**	.668**	.728**
Grit	18.42	4.02			-	.543**	.619**
Academic Self-Regulation	29.25	8.67				-	.763**
Academic Performance	6.7	1.8					-

Table 2. Measurement Model Output

Variable	CMIN/DF	CFI	IFI	TLI	RMSEA	AVE
Academic Motivation	1.346	.991	.992	.971	0.042	0.67
Academic Self Efficacy	1.482	.962	.956	.985	0.051	0.81
Grit	1.237	.923	.965	.975	0.049	0.73
Academic Self-Regulation	1.572	.901	.963	.936	0.053	0.76

Values of all the indices fall within the acceptable ranges (CMIN/DF <2, CFI ≥ .90, IFI ≥.95, TLI ≥0.95, RMSEA < 0.08 and AVE >.5) showing the adequacy of the measurement model.

Table 3. Structural Model Output

Fit Indices	CMIN/DF	CFI	IFI	TLI	NFI	RMSEA
Measured value	1.346	.991	.992	.971	.969	0.042

Table 4. Summary for Hypotheses Tests for Individual Parameter

Hypotheses	Paths	SEM Output			Results
		Standardised β	C.R. (t value)	P value	
H_1	Academic Self Efficacy→ Academic Motivation	.533	9.142	***	Supported
H_2	Academic Self Efficacy→ Academic Self-Regulation	.586	7.689	***	Supported
H_3	Grit→ Academic Self-Regulation	.120	1.575	.115	Not Supported
H_4	Grit→ Academic Motivation	.356	6.118	***	Supported
H_5	Academic Motivation→ Academic Performance	.435	9.529	***	Supported
H_6	Academic Self-Regulation→ Academic Performance	.525	11.505	***	Supported

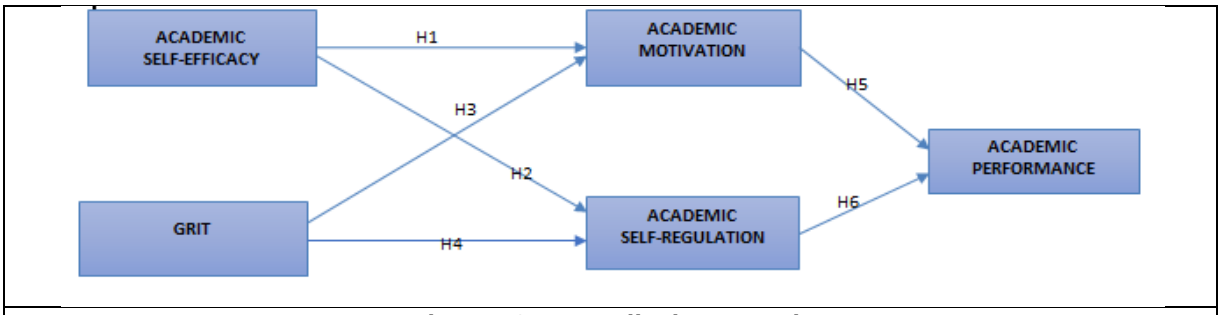

Figure 1. Conceptualized Framework

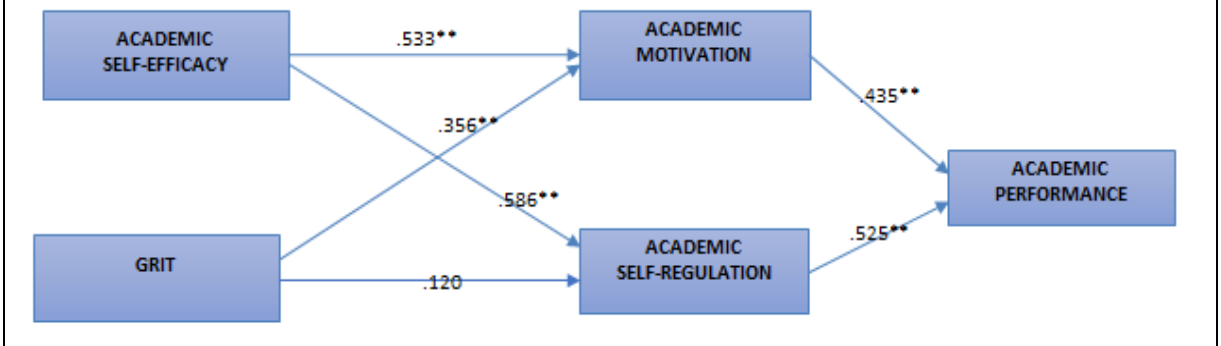

Figure 2. SEM Output

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RESEARCH ARTICLE

Assessment of Coastal Water and Air Quality of Haql, Gulf of Aqaba, Saudi Arabia

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ABSTRACT

Environmental assessment of Haql Governorate Coast was achieved by determining water quality of gulf of Aqaba of the Haql coast and air quality of the area. The analysis of water samples shows that levels of TDS, BOD, and COD were higher than the permissible limit. The nitrogen forms: total Kjeldahl nitrogen, NH_3, and NO_3^- and level of P and F^- were slightly higher than the threshold level. The total chlorine residual was found below the permissible limit. The concentration of metals was below to their permissible limit with the Hg ranked the highest while Cu the least. Therefore, metals can be arranged in the following order of their concentration: Hg > Pb > Ba > As >Se > Cd >Zn > Cu.Air quality monitoring was carried out by installing a mini station model (Aeroqual AQM60) at center of the area under investigation. Monitoring data recorded throughout the day (24 hours) for sampling of particulate matter (PM_{10}& $PM_{2.5}$) and gases (SO_2, NO_2, CO, H_2S, and VOCs). Air sampling was carried out from February 24 to March 12, 2020. The results show a sudden increase in PM_{10} during days 26-28 February and 6 March. The presence of the fifth event for PM_{10} and its absence for $PM_{2.5}$ indicates that the source of this event is mainly natural.

Keywords: HAQL COAST, METALS, PARTICULATE MATTER, POLLUTION, RED SEA

INTRODUCTION

The increasing population, coincided with economic growth, has been led to the urbanization and industrialization that adversely affected water, air, and soil [1], which manifest in the environmental pollution [2,3]. Marine ecosystem plays substantial role in regulating the natural course of the environment. The Red Sea coastline is exposed to a variety of anthropogenic activities such as oil spills, industrial wastewater and sewage, heated effluents of

desalination plants, explosives, building activities along the seashore, hypersaline water rejection, navigation and shipping operations, mining and raw material grinding, shipyards etc. In addition, human activities add metals, organic matter [4], hydrocarbons which adversely affect marine ecosystem. Therefore, excessive deposition of pollutants in the coastal areas degrades the quality of water that adversely affect the aquatic organisms and may lead to mass deaths and cessation of activities [5].

Besides marine ecosystem the quality of air, we breathe, directly affects human health. The burning of chemical fuels, emission of smoke from domestic stoves, and automobile exhaust have caused serious air pollution. Increased levels of occurrence of the gases such as ozone (O_3), carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen oxides (NO_x) are one of major contributors of air pollution. At present, anthropogenic air pollution is one of the biggest public health hazards worldwide [6] that accounts for about 4.2 million deaths per year [7]. Atmospheric aerosols are of critical importance because they affect the climate via direct and indirect radiative forcing that adversely impact the human health and ecosystem [8]. Particulate matter with an aerodynamic diameter of 2.5 micrometers or less ($PM_{2.5}$) and particulate matter with an aerodynamic diameter of 10 micrometers or less (PM_{10}) are the major causes of air pollution. Moreover, intensified anthropogenic emissions in the form of greenhouse gases viz. carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) act as the worst culprits of air pollution at local, regional and even at global scales [9-11].

The harmful effects of atmospheric pollutants on the human body vary greatly. For instance, $PM_{2.5}$ causes cardiovascular disease and lung cancer and affects air visibility and contributes to global climate change [12-14]. The $PM_{2.5}$ has the capacity to adsorb carcinogenic elements due to their great surface area and contains toxic elements such as hydrocarbons from combustion, and heavy metals from polluted environment. These particles have the ability to deteriorate the local and regional air quality, as well as the atmospheric visibility [15]. Moreover, the adverse effects of PM_{10} cannot be ignored, as it can affect visibility and temperature [13, 16]. Among the gaseous pollutants, CO is an extremely toxic pollutant. it passes through the respiratory system and enters the body's bloodstream [17], resulting in oxygen deprivation of the body tissues, with the most significant effects on the heart and brain [18,19]. Although, stratospheric O_3 is protecting us from the Sun's harmful radiations, however, ground-level O_3 is harmful to human health and is a pollutant [1].Ground-levelO_3stimulates the respiratory tract, triggers bronchitis and emphysema, and affects the nervous system of humans[20,21]. A higher risk of death from respiratory diseases is associated with increases in ozone [22].The greenhouse gas, CO influences the oxidization of the atmosphere via interactions with hydroxyl radical [23]. Similarly, N_2O in the atmosphere has a variety of toxicities and damages the bronchus and lungs after entering the human body, which can induce various types of respiratory inflammation [24]. In addition, SO_2 and NOx released from the combustion of fossil fuels or some types of industrial production are discharged into the atmosphere and undergo a chemical reaction to form sulfuric acid or nitric acid. Together with volatile organic compounds (VOCs), NOx is responsible for the formation of photochemical smog and ground-level O_3 in the presence of sunlight [16].

Atmospheric pollution in Saudi Arabia, as a serious consequence of the rapid economic and social growth associated with fuel over–consumption, has recently become of considerable interest. Numerous studies in Saudi Arabia are mostly concentrated in the urban regions of Jeddah [25-27] and Makkah [28-30]. Most of these studies reported poor air quality related to rapid urbanization, industrialization, and vehicular traffic. In contrast to studies in urban areas, studies on air pollutants and greenhouse gases at regional background sites not only provide valuable information on the influence of human activities on the environment and global change but also are helpful for understanding the transboundary transport of air pollution at a regional scale[31].

Therefore, the present study was carried out in the Haql governorate, located in the northern Tabuk province of Saudi Arabia. This study may be the first of its kind in Haql Governorate and even in Northern region of Saudi Arabia. In this study the water quality of gulf of Aqaba along Haql coast of Red Sea and air quality of the study area was assessed.

MATERIAL AND METHODS

Water sample collection

Water samples were collected near the water surface at a distance of about 6 meters from the shoreline from five points of gulf of Aqaba along the Haql coastal area of Red Sea, Saudi Arabia. Water quality was assessed using the mean values of five sampling data sets.

Analyses of water samples

Collected water samples were used for the estimation of following quality parameters: turbidity was measured using nephelometer, and pH using portable pH meter. Total dissolved solids (TDS) were estimated using conductivity meter. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were measured by the volumetric titration method [32]. Total Kjeldahl nitrogen (TKN) was estimated by colorimetric method [33], ammonia (NH_3) was estimated by Nessler method as described by Koch and McMeekin [34], nitrate (NO_3^-) was determined according to Rodger et al. [35] using cadmium reduction method [36]. Fluoride was estimated by SPADNS method adapted from standard methods for the examination of water and wastewater [37]. Total phosphorus (P) and total chlorine residual, and iron (Fe) were analyzed using standard methods [37]as described in method 8048-Hach, 8167-Hach, and 8008-Hach, respectively. Concentration of metals in water samples was estimated by inductively coupled plasma-atomic emission spectrometry as described in EPA-200.7 [38]. Concentration of following metals was tested: Arsenic (As), Cadmium (Cd), Copper (Cu), Mercury (Hg), Lead (Pb), Selenium (Se), Barium (Ba), and Zinc (Zn). The collected data were compared with permissible limits as recommended by Royal commission for environmental regulations (RCER), Saudi Arabia [39].

Analysis of air quality

Air quality monitoring was carried out by installation of one mini station model (Aeroqual AQM60, New Zealand) at center of the area under investigation. Monitoring data recorded throughout the day (24 hours) for sampling of PM_{10} and $PM_{2.5}$ and gases (SO_2, NO_2, CO, H_2S, VOCs). Air sampling was carried out from February 24, 2020 to March 12, 2020.

RESULTS AND DISCUSSION

Assessment of water quality of the Haql coastal area of Red Sea

Perusal of the data of water analysis (Table 1) shows that the turbidity level of the studied water samples was lower than the permissible limit [39]. Increased level of turbidity is an indicator of pollution that can significantly reduce water clarity. Elevated level of turbidity adversely affects aesthetic quality of water and can harm fish and other aquatic life by reducing food supplies, degrading spawning beds, and affecting gill function [40]. Phosphorus, through improving algal growth, is also considered as the key player in enhancing turbidity of water. In the present investigation increased value of P (Figure 2) coupled with a slight increase in BOD (Table 1) was observed but a lower level of turbidity was recorded. It indicates that the water is under oligotrophic phase. In addition, the pH of the water sample (Table 1) was almost closer to the threshold value (7.8 – 8.5) as recommended by RCER[39]. The pH of water is positively corelated with alkalinity and negatively corelated with CO_2 concentration, therefore, higher the pH, higher the alkalinity and lower the level of free CO_2 [41]. It is clear from Table 1 that the level of TDS exceeded the limit and BOD was also higher than the limit recommended by RCER. The TDS are the inorganic salts, organic matter, and other dissolved materials in water. Higher level of TDS was also reported by Ahmed et al. [42]. The increase in BOD indicates the presence of organic pollution that may be due to untreated domestic sewage, agriculture runoff, and residual fertilizers [43]. The data, exhibited in Table 1, show that the level of COD was much higher as compared with the limit recommended by RCER. Therefore, it can be concluded that the coastal waters of Red Sea in Haql region is under the adverse impact of anthropogenic activities.

The quality of water was also evaluated by analyzing the concentration of inorganic nitrogen, which was estimated in the form of TKN, ammonia and NO_3^-. It is evident from Figure 1 that collected water samples contain less than 2 mg/L of TKN (Figure 1) which was remarkably close to the permissible limit (RCER, 2004). Regarding, ammonia and NO_3^-, their concentration was higher (Figure 1) than the limit recommended by RCER. Among the various N forms, NO_3^- is the highly mobile species and is mainly responsible for N losses from soils [44].

The analyses of the data show higher total phosphorous content in the water samples as compared with the value recommended by RCER (Figure 2). Pollution of water bodies is mainly caused by excessive accumulation of N and P, released through the fertilizers [45]. The N, lost through evaporation, joins water bodies through atmospheric deposition. It is well known that NO_3^-, the highly mobile N species, is mainly responsible for N losses from soils through leaching [44]. On the other hand, P tightly binds with soil particles, thus, increase in soil erosion is the key factor that accelerates P runoff [46,47] to streams, rivers, lakes, and coastal regions. Phosphorus is considered as the primary limiting nutrient in eutrophication [48] and concentrations as low as between 10 and 20 µg P L^{-1}canpromote the growth of phytoplankton, aquatic plants, and algal blooms [49]. In the water samples, 0.071 mg/L of P was recorded (Figure 2) which is higher than the above-mentioned level of P that can encourage eutrophication. In eutrophic water bodies, owing to decomposition of organic matter, oxygen level is depleted that adversely affects aquatic ecosystem [50]. It is evident from the data (Figure 2) that water samples contain 1.78 mg/L of the fluoride which was higher than the recommended value of 1.5 mg/L. On the other hand, the level of residual chlorine and Fe was less than the permissible limit as recommended by RCER (Figure 2).

Increasing population coupled with industrial expansion has led to the accumulation of metals in the marine ecosystem which severely damages flora and fauna of aquatic ecosystem. Owing to their excessive accumulation, metals may enter the food chain and causes bio magnification thus adversely affect human health [51]. Metal-induced toxicity and health risks include kidney and skeletal damages, neurological disorders, endocrine disruption, cardiovascular dysfunction, and carcinogenic effects [52]. The data show that compared to the permissible limit the water samples contain lower value of all the studied metals viz. As, Cd, Cu, Hg, Pb, Se, Ba, and Zn (Figure 3). Therefore, in the water samples metals can be arranged in the following order of their concentration: Hg > Pb > Ba > As > Se > Cd > Zn > Cu. Our results corroborate the findings of Siddiqi et al. [53] and Fahmy et al. [54] who also reported lower level of metals in Red Sea water.

Air quality assessment of Haql city

Quality of air was assessed by measuring concentration of PM_{10} and $PM_{2.5}$ and gases such as CO, NO_2, SO_2, H_2S, and VOCs.

Particulate Matter (PM_{10} and $PM_{2.5}$) and gases

The time series of daily concentrations of PM_{10} & $PM_{2.5}$ are shown in Figure 4 and shows that five events of sudden increase in PM_{10} reach more than 860 % during days 26-28 February and 6 March. The presence of the fifth event for PM_{10} and its absence for $PM_{2.5}$ indicates that the source of this event is mainly natural. Among the analyzed gases, a significant variation was observed. The mean concentration (ppb) of oxides viz. CO, NO_2, and SO_2, was 0.280, 0.011, 0.019, respectively. Whereas level of H_2S, and VOCs was 0.012 ppb and 0.010 ppb, respectively. It is well known that fossil-fuels combustion and vehicular emissions are the key sources of emissions of CO, SO_2 and NO_X, respectively. Whereas oil and natural gas sector is the main source of VOCs and petroleum refineries are the major contributors of H_2S [55].These oxides serve as major indoor pollutants [56] which can cause tiredness, acute respiratory infections, chronic obstructive pulmonary disease, and lung cancer [57,58].

Statistics for PM_{10} and $PM_{2.5}$ at the study area in the period from 25 Feb to 12 Mar 2020 of the present study are shown in Table 2. The daily average concentration of PM_{10}ranged from 4.39 to 26.45 µg m^{-3} (with a mean value of 13.36 µg m^{-3}) and 3.08 to 18.83 µg m^{-3} (with a mean value of 9.17 µg m^{-3}) for $PM_{2.5}$.An hourly wind speed and direction of high concentrations of PM_{10} and $PM_{2.5}$ during different days of Feb and Mar is given in Figure 5 and 6.

are one of the main causes of air pollution and emissions of carbon from $PM_{2.5}$[59]. The impact of particulate matter on watery bodies is coupled with the existence of terrestrial and aquatic organisms and their productivity potential [60]. The concentration of PM_{10} and $PM_{2.5}$ was lower than the levels recommended by WHO. However, adverse health effects of exposure to $PM_{2.5}$ have been reported at lower levels [61].

Identification of the sources of PM_{10} and $PM_{2.5}$

Figure 6 A-C shows the wind rose of the prevailing wind speed and direction during days of high concentrations of PM_{10} and $PM_{2.5}$, where most of the wind is northeasterly predominance to the higher PM concentrations, also low concentrations with easterly and south-easterly winds. Wind rose, (Figure 5 A) shows that the period from 25 Feb -12 Mar 2020, 35% of the time the flow was north and northeasterly, 20% flow was northwesterly, 24% was southwesterly, and 21% was south and southeasterly. PM_{10} and $PM_{2.5}$ pollutant rose plots (Figure 5 B and C) show that the dominant wind direction is Northeasterly and is associated with a concentration range of 8-100 g/m^{-3} and fewer components were found in the easterly, south easterly with a concentration range of 8-20 $\mu g/m^{-3}$, while from northwesterly direction with a concentration range of 8-100 μgm^{-3}, respectively.

To get more detailed information about the source of pollutant substance and its distribution in the atmosphere at Haql city during monitoring period we need to track the pathways of these sources of contaminations using back trajectory to identify those sources of pollutions. Trajectories provide insightful information with regard to the regional and local emissions towards the observed levels of air pollution. The back trajectories of different days (25 Feb, 28 Feb, 3 Mar, 6 Mar, and 10 Mar 2020) are given in Figure 7 A-E, in which pollution levels are increased were chosen to determine the source of emissions. When emission levels for PM_{10} and $PM_{2.5}$ were measured we can notice that for each flow regime, a distinct path arose as shown in Figure 7 A-E. The northeast pathway of any trajectory consistently recorded the highest emission levels with PM_{10} and $PM_{2.5}$.At the other extreme, the east/southeast flow regime registered the lowest levels of emissions between these two flow regimes was the north/northwest flow regime that had the second highest levels of two pollutants, the southwest with the third highest level of the two pollutants.

In general, we can distinguish between three trajectories that were selected above Haql city (Figure 8). (1) North, which is associated with air masses passing through the desert, (2) North-East, where air comes from desert, and (3) from west, northwest and southwest where air masses pass through the red sea. Figure 7 D shows that trajectory labeled 1 (06Mar) which passes through red sea shows that apart from local pollution sources, there are sources of pollution located outside Haql City associated with passage of contaminants from outside regions. Almost all the high concentration 25-29 Feb and 6-8Mar are associated with air mass trajectories from the northeast. Although on 6 Mar afternoon the air stream from west (Figure 7 D), which set up air masses from Sinai Peninsula and the air masses passed over areas of high precursor emission source strength (dust storm appeared on satellite image on 7 March (Figure 8) and a noticeable change in the concentrations of PM_{10} & $PM_{2.5}$.The low concentrations in 1-4,6 Mar and 9 Mar tend to be associated with trajectories from the red sea and the absence of trajectories pass over land from the north

CONCLUSION

On the basis of assessment of results, it can be concluded that the water samples have the turbidity and pH level under the permissible limit. However, the concentration of TDS, BOD and COD was higher than their limit as recommended by RCER. The results show that the level of N and P was higher. Amon the metals, highest concentration of Hg was noticed while Cu was the least. However, metals were below the permissible limit. Regarding the air quality of the area, the daily average concentration of PM_{10} and $PM_{2.5}$ varied during different days of sampling and was associated with the hourly wind speed and direction. However, the values of PM_{10} and $PM_{2.5}$ were below the levels of the guidelines of USEP and WHO. Further studies ae required in the study area to

determine the usual direction of the gaseous pollutants and to identify the chemical composition and concentration of the particulate.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclose.

REFERENCES

1. Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglo E. environmental and health impacts of air pollution: a review. Front. Public Health 2020; 8, 14. 10.3389/fpubh.2020.00014
2. Heal MR, Kumar P, Harrison RM. Particles, air quality, policy and health. Chem. Soc. Rev. 2012; 41, 6606–6630.
3. Chen YY. Jin GZ, Kuamr N, Shi G. The promise of Beijing: Evaluating the impact of the 2008 Olympic Games on air quality. J Environ Econ Manage 2013; 66, 424–443.
4. Madkour HA, Dar MA. The anthropogenic effluents of the human activities on the red seacoast aturghada harbour (case study).Egypt J Aquat Res 2007; 33, 43–58.
5. Inyinbor AA, Adebesin BO, Oluyori, AP, Adelani-Akande TA, Dada AO, Oreofe T.A.Water pollution: effects prevention and climatic impact. In: Glavan, M., (Editor), Water Challenges of an Urbanizing World. 2018; Intech Open, 10.5772/intechopen.72018.
6. Yin Z, Cui K, Chen S, Zhao Y, Chao H, Chang-Chien G. Characterization of the Air Quality Index for Urumqi and Turfan Cities, China. Aerosol Air Qual Res 2019; 19, 282–306.
7. WHO. Air Pollution. 2021; Available online at: https://www.who.int/health-topics/air-pollution#tab=tab_1 (accessed on April 12, 2021).
8. Dahari N, Latif MT, Muda K, Hussein N. Influence of meteorological variables on suburban atmospheric PM2.5 in the southern region of peninsular Malaysia. Aerosol Air Qual Res 2020; 20, 14 – 25.
9. Lin W, Xu X, Sun J. Characteristics of gaseous pollutants at Jinsha, a remote mountain site in Central China. Sci China Ser B 2011; 41, 136–144.
10. Meng Z, Zhang R, Lin W, Jia X, Yu X, Wang G. Seasonal variation of ammonia and ammonium aerosol at a background station in the Yangtze River Delta region, China. Aerosol Air Qual Res 2014; 14, 756–766.
11. Thunis P, Miranda A, Baldasano JM, Blond N, Douros J, Graff A, Janssen S, Juda Rezler K, Karvosenoja N, Maffeis G, Martilli A, Rasoloharimahefa M, Real E, Viaene P, Volta M, White L.Overview of current regional and local scale air quality modelling practices: Assessment and planning tools in the EU. Environ Sci Policy 2016; 65, 13–21.
12. Matawle JL, Pervez S, Dewangan S, Shrivastava A, Tiwari S, Pant P, Deb MK, Pervez Y. Characterization of $PM_{2.5}$ source profiles for traffic and dust sources in Raipur, India. Aerosol Air Qual Res 2015; 15, 2537–2548.
13. Liang D, Wang YQ, Ma C, Wang YJ.Variability in transport pathways and source areas of PM_{10} in Beijing during 2009–2012. Aerosol Air Qual Res 2016; 16, 3130–3141.
14. Du PR, Du R, Lu ZD, Ren WS, Fu PQ. Variation of bacterial and fungal community structures in $PM_{2.5}$ collected during the 2014 APEC summit periods. Aerosol Air Qual Res 2018; 18, 444–455.
15. Cascio WE, Katwa LC, Linn WS, Stram DO, Zhu Y, Cascio JL, Hinds WC. Effects of vehicle exhaust in aged adults riding on Los Angeles freeways. Am J Respir Crit Care Med 2009; 179, 1175.
16. Muir D, Longhurst JWS, Tubb A. Characterization and quantification of the sources of PM_{10} during air pollution episodes in the UK. Sci Total Environ 2006; 358, 188–205.

17. Brauer M, Freedman G, Frostad J, Van Donkelaar A, Martin RV, Dentener F, Balakrishnan K. Ambient air pollution exposure estimation for the global burden of disease 2013. Environ Sci Technol 2016; 50, 79–88.
18. Amato F, Viana M, Richard A, Furger M, Prévôt ASH, Nava S, Lucarelli F, Bukowiecki N, Alastuey A, Reche C, Moreno T, Pandolfi M, Pey J, Querol X. Size and time-resolved roadside enrichment of atmospheric particulate pollutants. Atmos Chem Phys 2011; 11, 2917–2931.
19. Wei X, Liu Q, Lam KS, Wang T. Impact of precursor levels and global warming on peak ozone concentration in the Pearl River Delta Region of China. Adv Atmos Sci 2012; 29, 635–645.
20. Zhang Y, Wang Y. Climate-driven ground-level ozone extreme in the fall over the Southeast United States. Proc Natl Acad Sci USA. 2016; 113, 10025–10030. doi: 10.1073/pnas.1602563113
21. Zhang J, Wei Y, Fang Z. Ozone pollution: a major health hazard worldwide. Front Immunol 2019; 10, 2518. 10.3389/fimmu.2019.02518
22. Jerrett M, Burnett RT, Pope CA 3rd, Ito K, Thurston G, Krewski D, Shi Y, Calle E, Thun M. Long-term ozone exposure and mortality. N Engl J Med 2009; 360, 1085-1095.
23. Gligorovski S, Strekowski R, Barbati S, Vione D. Environmental implications of hydroxyl radicals (•OH). Chem Rev 2015; 115, 13051–13092.
24. Tong X, Wang B, Dai WT, Cao JJ, Ho SSH, Kwok TCY, Lui KH, Lo CM, Ho, KF. Indoor air pollutant exposure and determinant factors controlling household air quality for elderly people in Hong Kong. Air Qual Atmos Health 2018; 11, 695–704.
25. Khodeir M, Shamy M, Alghamdi M, Zhong M, Sun H, Costa M, Chen LC, Maciejczyk P. Source apportionment and elemental composition of $PM_{2.5}$ and PM_{10} in Jeddah City, Saudi Arabia. Atmos Pollut Res 2012; 3, 331–340.
26. Alghamdi MA, Almazroui M, Shamy M, Redal MA, Alkhalaf AK, Hussein MA, Khoder MI. Characterization and elemental composition of atmospheric aerosol loads during springtime dust storm in western Saudi Arabia. Aerosol Air Qual Res 2015; 15, 440–453.
27. Nayebare SR, Aburizaiza OS, Khwaja HA, Siddique A, Mirza M, Hussain MM, Zeb J, Khatib F, Carpenter DO, Blake D.R. "Chemical Characterization and Source Apportionment of PM2.5 in Rabigh, Saudi Arabia", Aerosol Air Qual Res 2015; 16, 3114–3129.
28. Al-Jeelani HA. Air quality assessment at Al Taneem area in the Holy Makkah City, Saudi Arabia. Environ Monit Assess 2009a; 156, 211–222.
29. Al-Jeelani HA. Evaluation of air quality in the Holy Makkah during Hajj season 1425 H. J Appl Sci Res 2009b; 5, 115–121.
30. Munir S, Habeebullah TM, Seroji AR, Gabr SS, Mohammed AMF, Morsy EA. Modeling particulate matter concentrations in Makkah, applying a statistical modeling approach. Aerosol Air Qual Res 2013; 13, 901–910.
31. Cheng L, Ji D, He J, Li L, Du L, Cui Y, Zhang H, Zhou L, Li Z, Zhou Y, Miao S, Gong Z, Wang Y. Characteristics of Air pollutants and greenhouse gases at a regional background station in Southwestern China. Aerosol Air Qual Res 2019; 19, 1007–1023.
32. APHA (American Public Health Association), (1995). Standard Methods for Analysis, America Public Health Association.
33. EPA Nitrogen, Kjeldahl, Total (Colorimetric, Automated Phenate) by Autoanalyzer, Method 351.1, 1978; U.S. Environmental Protection Agency, Washington, D.C.
34. Koch FC, McMeekin TL. "A new direct nesslerization micro-kjeldahl method and a modification of the nessler-folin reagent for ammonia." J. Am. Chem. Soc. 1924; 46, 2066–2069.
35. Rodger BB, Andrew DE, Eugene WR. Standard methods for the examination of water and wastewater. APHA, 2017; Washington, DC, 23rd edition.
36. Hach Company, Nitrate. Cadmium reduction method, Method 8039 (DOC316.53.01066), Water Analysis Handbook, 2014; Hach Company, Loveland, CO, Edition 9.
37. APHA (American Public Health Association) Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998; American Public Health Association Inc., Washington.
38. EPA"Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry," 1994; Revision 4.4. Cincinnati, OH.

39. R.C.E.R. Royal Commission Environmental Regulations, 2004, Vol. 1.
40. MPCA. Minnesota Pollution Control Agency, 2008.
41. Mackereth FJH, Heron J, Talling JF, Water analysis. Fresh Water Biological Association, 1989; UK.
42. Ahmed SM, Hussain M, Abderrahman W. Using multivariate factor analysis to assess surface/logged water quality and source of contamination at a large irrigation project at Al-Fadhli, Eastern Province, Saudi Arabia. Bull Eng Geol Environ 2005; 64, 319–327.
43. Maurya PK, Malik DS, Yadav KK, Kumar A, Kumar S, Kamya H.Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. Toxicol Rep 2019; 6, 472–481.
44. Tesoriero A, Liecscher H, Cox S.Mechanism and rate of denitrification in an agricultural watershed: electron and mass balance along ground water flow paths. Water Resour Res 2000; 36, 1545–1559.
45. Khan MN, Mobin M, Abbas ZK, Alamri SA.Fertilizers and their contaminants in soils, surface and groundwater. In: The Encyclopedia of the Anthropocene, DellaSala DA. Goldstein MI. (Editros), Oxford: Elsevier, 2018; vol. 5, p. 225-240.
46. Uusitalo R, Yli-Halla M, Turtola E.Suspended soil as a source of potentially bioavailable phosphorus in surface runoff waters from clay soils. Water Res 2000; 34, 2477–2482.
47. Eghball B, Gilley JE. Phosphorus risk assessment index evaluation using runoff measurements. J Soil Water Conserv 2001; 56, 202–206.
48. Correll DL. The role of phosphorus in the eutrophication of receiving waters: a review. J EnvironQual 1998; 27, 261–266. https://doi.org/10.2134/jeq1998.00472425002700020004x
49. Powlson DS.Phosphorus, agriculture and water quality. Soil Use Manag 1998; 14, 123. https://doi.org/10.1111/j.1475-2743.1998.tb00629.x.
50. Pickney JL, Paerl HW, Tester P, Richardson TL.The role of nutrient loading and eutrophication in estuarine ecology. Environ Health Perspect 2001; 109, 699–706.
51. Ashraf W, Seddigi Z, Abukibash A, Khalidi K.Level of selected metals in canned fish consumed in Kingdom of Saudi Arabia. Environ Monit Assess 2006; 117, 271–279.
52. Renieri EA, Safenkova IV, Alegakis AK, Slutskaya ES, Kokaraki V, Kentouri M, Dzantiev BB, Tsatsakis AM. Cadmium, lead and mercury in muscle tissue of gilthead seabream and seabass: risk evaluation for consumers. Food Chem Toxicol 2019; 124, 439–449.
53. Siddiqi, Z.M., Saleem, M., Basheer, C., (2016). Surface water quality in a water run-off canal system: A case study in Jubail industrial city, Kingdom of Saudi Arabia.
54. Fahmy MA, Fattah LMA, Abdel-Halim AM, Aly-Eldeen MA, Abo-El-Khair EM, Ahdy HH, Hemeilly A, El-Soud AA, Shreadah MA. Evaluation of the quality for the egyptian red sea coastal waters during 2011-2013. J Environ Protec 2016; 7, 1810-1834. http://dx.doi.org/10.4236/jep.2016.712145.
55. Salman A, Al-Tayib M, Hag-Elsafi S, Zaidi FK, Al-Duwarij N. Spatiotemporal assessment of air quality and heat island effect due to industrial activities and urbanization in southern Riyadh, Saudi Arabia. Appl Sci 2021; 11, 2107. https://doi.org/10.3390/ app11052107
56. Zhang H, Srinivasan R. A systematic review of air quality sensors, guidelines, and measurement studies for indoor air quality management. Sustainability 2020;12, 9045. 10.3390/su12219045
57. Ghaffarianhoseini A, AlWaer H, Omrany H, Ghaffarianhoseini A, Alalouch C, Clements-Croome D, Tookey J. Sick building syndrome: are we doing enough. Archit Sci Rev 2018; 61, 99–121.
58. Smith KR. National burden of disease in India from indoor air pollution. Proc. Natl. Acad. Sci. USA 2000, 97, 13286–13293.
59. BianQ, Alharbi B, Shareef M, Husain T, Pasha M, Atwood S, Kreidenweis S. Sources of $PM_{2.5}$ carbonaceous aerosol in Riyadh, Saudi Arabia. Atmos Chem Phys 2018; 18, 3969–3985.
60. Zuhara S, Isaifan R. The impact of criteria air pollutants on soil and water: a review. J Environ Sci Poll Res 2018; 4, 278–84. 10.30799/jespr.133.18040205

61. Crouse DL, Peters PA, van DonkelaarA, Goldberg MS, Villeneuve PJ, Brion O, Khan S, Atari DO, Jerrett M, Pope CA, Brauer M, Brook JR, Martin RV, Stieb D, Burnett RT. 'Risk of nonaccidental and cardiovascular mortality in relation to long-term exposure to low concentrations of fine particulate matter: a Canadian national-level cohort study', Environ Health Perspect 2012; 120, 708–714

Table 1. Physicochemical properties of water of Gulf of Aqaba, Haql coast, Saudi Arabia

Physicochemical characteristics				
Turbidity (NTU)	**pH**	**TDS (mg/L)**	**BOD (mg/L)**	**COD (mg/L)**
0.28 ± 0.017	8.2 ± 0.58	39205 ± 11.42	29 ± 2.71	3166 ± 17.05

Values are average ± SE of five independent replicates. TDS: total dissolved solids; BOD: biochemical oxygen demand; COD: chemical oxygen demand

Table 2. Overall data on air quality in the period from 25 February to 12 March 2020 at Haql city

Pollutant	Mean	S.D.	Min.	Max.
PM_{10} (µg m^{-3})	13.36	7.23	4.39	26.45
$PM_{2.5}$ (µg m^{-3})	9.17	4.61	3.08	18.83
CO (ppb)	0.280	0.200	< 0.040	0.570
NOx (ppb)	0.011	0.006	0.003	0.025
SO_2 (ppb)	< 0.009	0	< 0.009	< 0.009
H_2S (ppb)	< 0.012	0	< 0.009	< 0.009
VOCs (ppb)	< 0.010	0	< 0.010	< 0.010
S.D. = standard deviation, Min. = Minimum, Max. = Maximum				

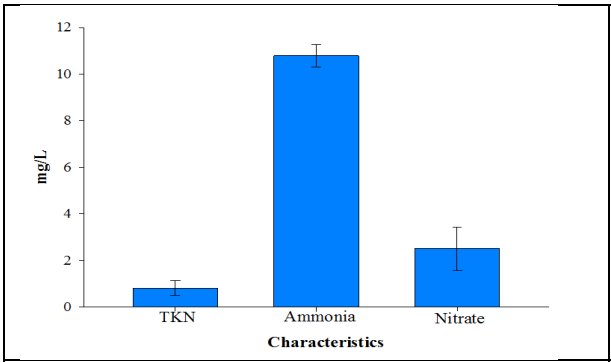

Figure 1. Total Kjeldahl nitrogen (TKN), ammonia and nitrate in the water of Gulf of Aqaba, Haql coast, Saudi Arabia. An average of five determinations is presented, with bars indicating SE

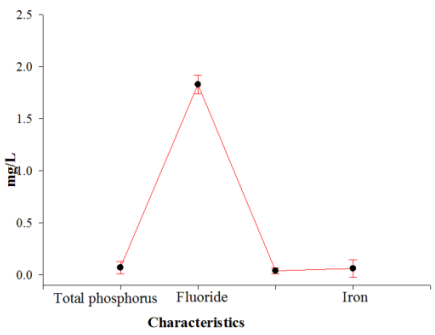

Figure 2. Total phosphorous, fluoride, total chlorine residual and iron in the water of Gulf of Aqaba, Haql coast, Saudi Arabia. An average of five determinations is presented, with bars indicating SE.

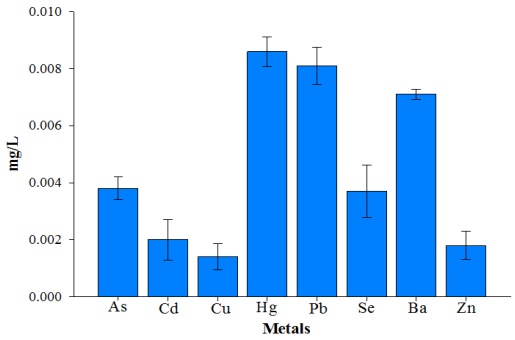

Figure 3. Concentration of metals in the water of Gulf of Aqaba, Haql coast, Saudi Arabia. An average of five determinations is presented, with bars indicating SE

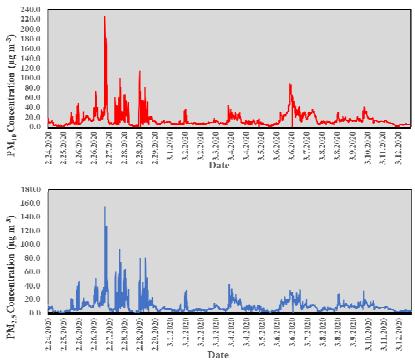

Figure 4. Time series plot of observed $PM_{10}$and $PM_{2.5}$ from 25 February to 12 March 2020.

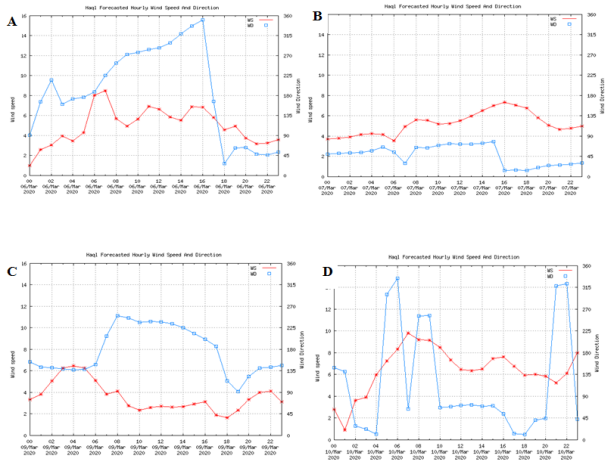

Figure 5. Hourly forecasted wind speed and direction during days (6, 7, 9, and 10 March 2020) of high concentrations of PM_{10} and $PM_{2.5}$

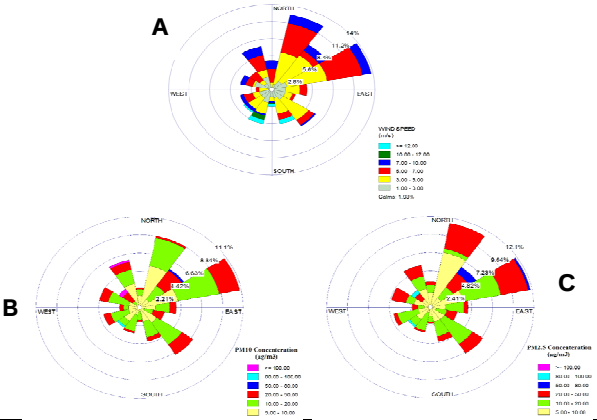

Figure 6. Wind rose chart of Haql city from 25 Feb – 12 Mar 2020. (A) Wind rose diagram of wind speed and direction, (B) PM_{10} rose for PM_{10} concentrations and wind direction, and (C)$PM_{2.5}$ rose for $PM_{2.5}$ concentrations and wind direction

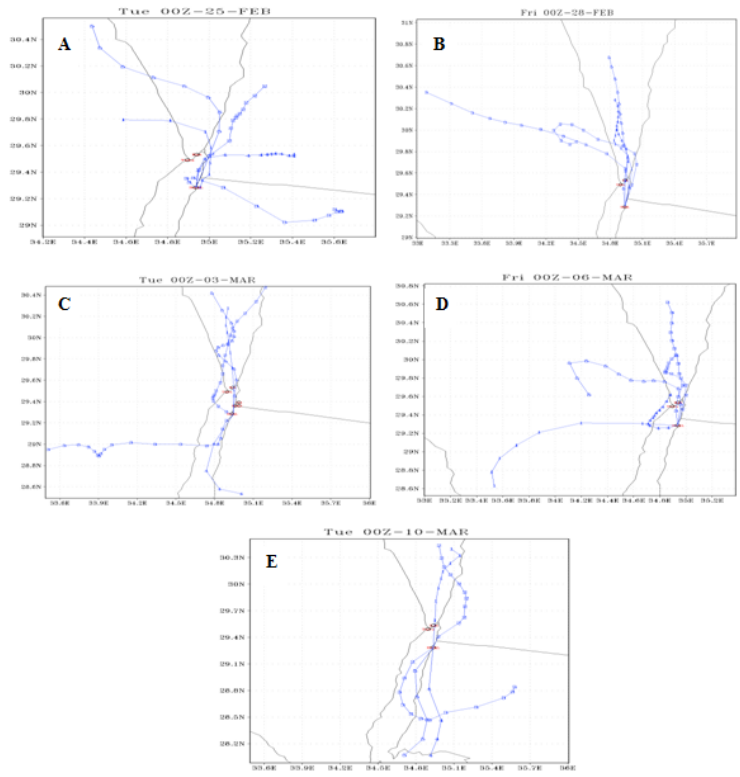

Figure 7. (A-E).24-hour forecasted back trajectories for different days reached Haql city at 08:00 AM. trajectory labeled A to E refer to first to fifth days forecasted back trajectories started from initial Tim 25 February 2020

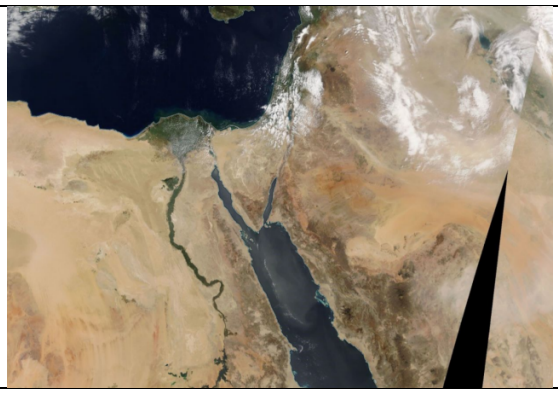

Figure 8.Satellite image of 07 March 2020 shows a haze resulting from sand storm over Sinai Peninsula

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RESEARCH ARTICLE

An *In Silico* Strategy to Assess the Antibacterial Activity of Phytochemicals from Fenugreek (*Trigonella foenum- graecum*) against E. coli

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ABSTRACT

Antibiotic resistance is not only a challenge to health workers but also a threat to public health. Extensive research has been proved that rising antibiotic resistance in multi drug resistance gram negative bacteria is a global concern. Infection caused by multidrug resistant (MDR), extensively drug resistant (XDR) and pan drug resistant (PDR) strains of *E.coli* has created a serious problem worldwide. To overcome this problem, researchers are trying to find an alternative approach. Plant derived phytochemicals are safe, less side effect, biological origin, low cost, less toxic and readily available with potential therapeutic values. Phosphoenolpyruvate carboxylase (PEP carboxylase) is the primary enzyme involved in energy production in *E.coli* bacteria. In aerobic condition, carboxylation of phosphoenolpyruvate is catalyzed by phosphoenolpyruvate carboxylase (PEP carboxylase). Hence, inhibition of PEP carboxylase activity blocks the energy production pathway in *E.coli*. PEP carboxylase could be a potential target to inhibit the E.coli and to suppress the E.coli infection. In this present study phosphoenolpyruvate carboxylase (PEP carboxylase) protein structure (PDB ID 1JQN) of *E.coli* was retrieved from PDB site and used as target protein. Phytochemicals of *Trigonella foenum- graecum* (Fenugreek) scopoletin structure was also retrieved from Pubchem. Autodock software was used to study the interaction between PEP carboxylase and scopoletin. We concluded that, phytochemical scopoletin of *Trigonella foenum- graecum* (Fenugreek) is effective against PEP carboxylase enzyme of E.coli. It can be used for drug designing and vaccine development.

Keywords: Phytochemical, Autodock, *Trigonella foenum- graecum* (Fenugreek), Phosphoenolpyruvate carboxylase (PEP carboxylase), *E.coli*, scopoletin.

INTRODUCTION

Escherichia coli are gram negative bacteria of family Enterobacteriaceae. *E.coli* is a commensal natural gut micro flora of human and other endothermic animal. Different intestinal and extraintestinal infection including UTI, sepsis and bacterimia are caused by *E.coli* [1]. The high mortality and morbidity rate due to *E.coli* infection are great concern now a day. Emerging multidrug resistance *E.coli* strains are very challenging to treat in this antibiotic era. Studies have found that, extended spectrum beta lactamase (ESBL) producing *E.coli* are present both in human and non-human sources [2]. A systematic review have declared that, approximately 44% *E.coli* infection are community acquired, 27% are health care associated and 27% are hospital acquired [3]. In this current time period, the rising cases of *E.coli* infection due to multidrug resistance *E.coli* bacteria are a major challenge for health care professionals [4, 5]. This global issue becomes a focused area for researcher to find an alternative way to treat the infection caused by multidrug resistance *E.coli* bacteria.

Phosphoenolpyruvate (PEP) is an essential precursor for the synthesis of succinic acid and plays an important role in the metabolism of *E. coli* [6]. In aerobic condition, carboxylation of phosphoenolpyruvate is catalyzed by phosphoenolpyruvate carboxylase (PEP carboxylase) and involved in energy production [7]. Inhibition of PEP carboxylase directly impacts the metabolism of *E.coli* as well as energy production. This inhibition may leads to the lysis of bacterial cell. In view to this, PEP carboxylase is the target for the inhibition of *E.coli.* It can be a good alternative approach to design a drug for the treatment of life threatening disorder caused by multidrug resistance *E.coli* bacteria.

Trigonella foenum- graecum (Fenugreek) is a popular annual herb plant. Fenugreek is native to Eastern Europe and some part of Asia but due to its various pharmacological and therapeutic benefits, it is cultivated in all over the world. More positive impacts on health and no or less side effects make it more popular all over the world [8]. Following is the taxonomical classification of *Trigonella foenum- graecum* (Fenugreek) [9].

Taxonomy
Kingdom: Plantae
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Genus: *Trigonella*
Fenugreek seeds are most commonly used spices in India. Leaves are also used as green vegetables. Extensive uses of Fenugreek seeds and leaves as a phytomedicine are reported worldwide [10]. An enormous pharmacological property of Fenugreek includes antidiabetic, anticancer, antimicrobial, anti fertility activity etc [11]. In this present study, molecular docking was performed by using Autodock Vina. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules. Docking is frequently used to predict the binding orientation of small molecule (ligand) drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule [12].

MATERIAL AND METHODS

phosphoenolpyruvate carboxylase (PEP carboxylase) protein structure (PDB ID 1JQN) of *E.coli* was retrieved from PDB site containing resolution about 2.35A^0 . This is the target protein used in this molecular docking study. Phytochemicals of *Trigonella foenum- graecum* (Fenugreek) scopoletin structure was also retrieved from Pubchem. To screen the binding affinity of phytochemicals with target protein molecular docking study was performed by using Autodock software. The value was obtained in terms ΔG Kcal/mol. Energy value is inversely proportional to the

affinity of the molecule to be used as a drug. So, lesser the (ΔG) of phytochemical molecule indicates higher affinity of that phytochemical as a drug.

RESULTS AND DISCUSSION

From the Autodock molecular docking studies, a good ΔG Kcal/mol value was obtained for the compound scopoletin. The binding affinity of different small compounds (ligand) with PEP carboxylase protein ranges from -6.3 Kcal/mole to -7.1 Kcal/mole (as shown in table no 1). This lesser value of ΔG indicates the successful approach of phytochemical scopoletin from Fenugreek (*Trigonella foenum- graecum)* as a drug against emerging bacterial pathogen *E. coli* phosphoenolpyruvate carboxylase (PEP carboxylase) enzyme. This can be an alternative approach for the treatment of various disorder caused by *E.coli*.

CONCLUSION

This in-silico study concluded that scopoletin, phytochemicals from Fenugreek (*Trigonella foenum- graecum)* can be used as a potent inhibitor molecule against *E.coli* pathogen. In vitro study can be used as a very basic approach to design a drug against many emerging pathogens. Further in-vitro and in-vivo studies are required to use the scopoletin as a potent drug against the *E.coli* infection. Use of bioinformatics tool and computational biology for the drug design is a better approach to limit the time and cost that is required for the in-vitro and in-vivo drug designing studies.

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REFERENCES

1. B Marc, Johnson JR, Biggelaar AHJ, Georgalis L, Geurtsen J et al. Epidemiology of Escherichia coli Bacteremia: A Systematic Literature Review. Clinical infectious diseases, 2020; 1-9.
2. Hu Y, Matsui Y, Riley L W. Risk factors for fecal carriage of drug-resistant Escherichia coli: a systematic review and meta-analysis. Antimicrobial Resistance and Infection Control, 2020; 9-31.
3. Montassier E, Batard E, Gastinne T, Potel G, Cochetiere MFL. Recent changes in bacteremia in patients with cancer: a systematic review of epidemiology and antibiotic resistance. European Journal of Clinical Microbiology & Infectious Diseases, 2013; 32: 841-850.
4. Erb A, Sturmer T, Marre R, Brenner H. Prevalence of antibiotic resistance in Escherichia coli: overview of geographical, temporal, and methodological variations. European Journal of Clinical Microbiology & Infectious Diseases , 2007; 26: 83-90.
5. Alizade H. *Escherichia coli* in Iran: An Overview of Antibiotic Resistance: A Review Article. Iranian Journal of Public Health, 2018; 47:1-12.
6. Tan Z, Zhu X, Chen J, Li Q, Zhang X. Activating Phosphoenolpyruvate Carboxylase and Phosphoenolpyruvate Carboxykinase in Combination for Improvement of Succinate Production. Applied and Environmental Microbiology, 2013; 79: 4838-4844.
7. Millard C S, Chao Y P, Liao J C, Donnelly M I. Enhanced Production of Succinic Acid by Overexpression of Phosphoenolpyruvate Carboxylase in Escherichia coli. Applied and Environmental Microbiology, 1996; 62: 1808-1810.

8. Yadav U C S, Baquer N Z. Pharmacological effects of Trigonella foenum-graecum L. in health and disease. Pharmaceutical Biology, 2014; 52(2): 243-254.
9. Gupta S, Gupta P, Patsariya S. Phytochemical and Pharmacological review on Fenugreek (Trigonella foenum-graecum). Current research in pharmaceutical sciences, 2012; 1:11-13.
10. Adejuwon O M, Matthew K O, Ishaya F A, Warra A A, Adedara A O. Physico-chemical, Phytochemical and Antimicrobial Analysis of Black cumin and Fenugeek seed oils. International Journal of Engineering Applied Sciences and Technology, 2020; 5: 124-128.
11. Mallik S, Sharangi A B, Sarkar T. Phytochemicals of Coriander, Cumin, Fenugreek, Fennel and Black Cumin: A Preliminary Study. Springer, 2020.
12. Ismail S Y, Uzairu A. In silico QSAR and molecular docking studies of sulfur containing shikonin oxime derivatives as anti-cancer agent for colon cancer. Radiology of infectious diseases, 2019;6:108-121.

Table 1. Compounds and their binding energy value in Kcal/mole

Name of the compound	Binding energy value in K/Cal (ΔG)
C1	-7.1
C2	-6.9
C3	-6.9
C4	-6.7
C5	-6.7
C6	-6.7
C7	-6.6
C8	-6.3
C9	-6.3

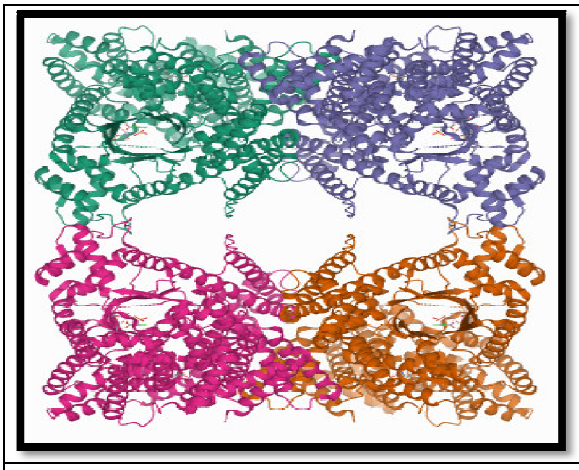

Figure 1- PDB ID-1JQN (Protein Phosphoenol pyruvate carboxylase of *E.coli*)

Figure 2- Docking of 1JQN (PEP Carboxylase) of *E.coli* with Autodock

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RESEARCH ARTICLE

Quality Determination and Acceptability of Fishes of the Nanpura Fish Market, Surat (Gujarat)

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ABSTRACT

Aquaculture provides majority of the world's need of food and one of the richest sources of nutrition. Freshness is the major concern when it comes to fishes as fish and fish products are highly perishable with less shelf-life. Sensory methods for quality assessment of the fish are rapid and efficient and they analyze the quality on the basis of changes in sensory attributes such as taste, texture and odour of skin, mucus, gills, eyes, flesh etc. In present study freshness evaluation of Indian major carp fishes sold in the Nanpura fish market of Surat, Gujarat was carried out using European Union Scheme (EU Scheme) and Quality Index Method (QIM). Comparative analysis of freshness of fishes was carried out between fishes sold in well-furnished shop and road vendors. Rapidly increased QI score in fishes of road vendors was recorded compared to fishes at shop. It was observed that good infrastructure, proper storage conditions and good hygienic conditions can help in increasing the shelf-life of fish slowing down the deterioration of fishes.

Keywords: Aquaculture, fishes, nutrition, taste, texture.

INTRODUCTION

Major carps are important species that support freshwater fisheries of India mainly due to its high commercial value, the taste and appearance of meat [1]. It is estimated that around 60% of people in many developing countries depend on fish for over 30% of their animal protein supplies, while almost 80% in most developed countries obtain less than 20% of their animal protein from fish [2]. Fish are good source of high quality protein,omega-3 fatty acids, vitamins (vitamin D, B12 and B6) and minerals such as Calcium, Phosphorus, Iron, Zinc, Iodine, Magnesium and Potassium [3]. Fish and fishery products are perishable and it is the major concern in fish industries all over the world [4].Quality of fish is based upon freshness and taste because both are essential and vital for aquaculture sector as well as consumers. Freshness and quality deteriorates easily if fishes are not handled properly [5,6,7] Quality of seafood

may be influenced by improper handling, processing and mistreatment during storage which leads to changes in sensory characteristics that may lower its price value[8]. Considering that consumer preferences it is essential to determine the quality of fish in terms of taste, texture and odor, in order to avoid losing customers and result ant financial loss. Many of research studies are available on methods to determine the freshness of fish and estimate product shelf-life using sensory methods of assessment. US NOAA Seafood Inspection Program [9].European Union (EU) grading scheme described in the Council Regulation (EC) No. 2406/96 [10]the Quality Index Method [11]are the most commonly used sensory methods for the freshness and quality assessment of raw fish in the inspection service and fishing industry. The present study was conducted to assess the quality changes in Indian major carp fishes stored in well-furnished shop as well as road side vendors of wholesale fish market of Nanapura, Surat. Sensory methods were used to access the fish quality and it was intended to monitor the changes occurring during selling hours.

METHODOLOGY

Nanpura wholesale fish market of Choryasi taluka of Surat District was selected as study site. Samples selection for the study was based on their storage conditions during selling hours. Two different categories on the basis of storage facilities while selling were selected for study. First category selected was well furnished shop which has ice storage facilities and proper hygienic condition while other was road side venders who sell fishes in unhygienic condition without proper storage facilities. For analysis by sensory methods, samples were analyzed twice a day. First when fresh fishes arrived when market opened and lastly in leftover fishes at closing time for quality determination using sensory methods. This observational study was carried out on daily basis for period of a month. The sensory assessment was conducted using the Quality Index Method (QIM) originally developed by the Tasmanian Food Research unit 9[11]and EU grading scheme [European Union Council Regulations (EC) No 2406/96][12] respectively. According to the European Union scheme, three grades of freshness are established considering different sensory attributes: E, A and B, corresponding to various stages of spoilage. E (Extra) is the highest possible quality, while below B is the level where fish is considered unfit for human consumption.

QIM is based on the significant sensory parameters for raw fish when using many parameters and a score system from 0 to 4 demerit points. QIM uses a practical rating system, in which the fish is inspected and the fitting demerit point is recorded. The scores for all the characteristics are then summed to give an overall sensory score, the so-called quality index. Following the system very fresh fish was given the score zero while increasingly larger totals resulted as fish being deteriorated.

RESULTS AND DISCUSSION

The frequency of recorded sensory parameters during initial and final observations of fishes at shop as well as road side vendors are presented in Table 1. At the time of arrival in market, majority of the fishes arrived at both shop and the road side vendors were of excellent quality with bright, iridescently pigmented skin, aqueous and transparent mucus, bright colored eyes and firm, elastic, smooth surfaced flesh. At the end of selling hours, rapidly progressed deterioration in sensory attributes were observed in fishes belong to road side vendors with no proper storage conditions. In the shops where fishes stored in ice and proper hygienic condition, freshness and quality were remained excellent to acceptable. Most affected and perished areas were skin, gills and flesh. Similar observations were recorded by Lanzarin and workers [13]where they found skin color and odour to be the most influencing attributes that affects the QI score of fresh gutted Amazonian Pintado. The changes in Quality index score for Indian major carp is shown in Table-2. QI score of samples from shop was almost same at initial and end of the sell. There was a large difference in QI score of road side vendors' samples at initial observations and end time observations of the sell. Difference in QI score indicated quality deterioration of fish which could be due to lack of proper preservation facilities and hygiene. Considerable observational changes in fish were: less pearl shiny skin, milky

clotted mucus; fish smelled like cucumber and metal. dark grey to matt grey eyes pupils, light brown to grey brown gills, and mucus color from milky to brown and smelled like sour and metal. Flesh of fish was slightly soft and pinkish. QI score observed to be increased from 2.86 t0 7.26 at the closing time at road vendors whereas in shop the QI score was slightly increased from 3 to 3.58 (Table-2).Similar results were observed by Campus and co-workers[14].where he evaluated the effect of MAP packed farmed gilthead sea bream *(Sparus aurata)* on QIM scores at different temperatures and stated that temperatures above 8°Cdrastically accelerated the rate of increase in QI score.

Moreover, it was observed that at the arrival time, QI score was recorded high in fishes of shop as compared to road side vendors which indicated possibility of leftover fishes of previous day in shop. Due to proper storage conditions and maintained hygiene, the quality and freshness of fishes could be preserved. Poor storage facilities and unhygienic conditions under open air deteriorate fishes rapidly and decreases shelf-life which is the reason of poor quality of fishes at road side vendors during market closing time.

CONCLUSION

Sensory methods for evaluation of freshness and quality of fishes appear to be easy, efficient and rapid methods. Following these methods, the present study suggests the requirement of storage facilities provision as well as better infrastructure to maintain the hygienic condition in market to fulfill consumers' needs and to provide them good quality fishes.

REFERENCES

1. Dhanapal, K., Sravani, Balasubramanian, GVS Reddy . Quality determination of rohu (*Labeorohita*) during ice storage. Tamilnadu J. Veterinary & Animal Sciences, 9(2), pp. 146-152,2013.
2. Shady, M., Ali Abd El-Fatah, Hamed 'Quality Attributes of the Most Common Consumed Fresh Fish in Saudi Arabia', International Journal of Nutrition and Food Sciences, 5(2):85-94, 2016
3. Naziba, K.T, Asifur, R.S., Amira M.A., Israt J.M. 'A Review on the Techniques for Quality Assurance of Fish and Fish Products', International Journal of Advanced Research in Science, Engineering and Technology, 4: 4190-4205, 2017.
4. Chebet L. "Rapid" (alternative) methods for evaluation of fish freshness and quality [Msc Thesis]. Akureyri, Iceland: University of Akureyri; 2010.
5. Cheng, J.H., et al.: Recent advances in methods and techniques for freshness quality determination and evaluation of fish and fish fillets: a review. Crit. Rev. Food Sci. Nutr. 55(7), 1012–1225 ,2015
6. Dabadé, D.S., et al.: Prediction of spoilage of tropical shrimp (Penaeus notialis) under dynamic temperature regimes. Int. J. Food Microbiol. **210**, 121–130 ,2015
7. Du, L., et al.: A model for discrimination freshness of shrimp. Sens. Bio-Sens. Res. **6**, 28–32 2015
8. Solanke, S.S., et al.: Production, price spread and marketing efficiency of farmed shrimp in Thane District of Maharashtra. Indian J. Fish. **60**(3), 49–50 ,2013
9. NOAA, NOAA Seafood Inspection Program [WWW Document]. URL https://www.fisheries.noaa.gov/insight/noaas-seafood-inspection-program (accessed 4.18.20) , 2017.
10. Anonymous, Council Regulation (EC) No 2406/96 of 26 November 1996 laying down common marketing standards for certain fishery products. Off. J. Eur. Communities 334, 1–15, 1996
11. Bremner, H.A., A convenient, easy to use system for estimating the quality of chilled seafoods. Fish Process. Bull. 7, 59–70, 1985.
12. European Union (1996) Council Regulations (EC) No 2406/96 of 26 November Laying down common marketing standards for certain fishery products. Official Journal of the European Communities 1996, No L-334/2, 1996.

13. Lanzarin M, Ritter DO, Novaes SF, Monteiro MLG, Almeida Filho ES, Marsico ET, Franco RM, Conte Jr CA, Freitas MQ). Quality Index Method (QIM) for ice stored gutted Amazonian Pintado (*Pseudoplatystoma fasciatum*x *Leiarius marmoratus*) and estimation of shelf life. LWT - Food Sci. Technol. 65:363-370, 2016.
14. Campus M, Bonaglini E, Cappuccinelli R, Porcu MC, Tonelli R, Roggio T. Effect of modified atmosphere packaging on Quality Index Method (QIM) scores of farmed gilthead seabream (*Sparus aurata*L.) at low and abused temperatures. *J Food Sci.*; 76(3):185-191,2011.

Table:1 Criteria for European Union Scheme for Shop and road side vendors

Term		Shop				Road side vendors			
		Initial		End		Initial		End	
		Number	%	Number	%	Number	%	Number	%
Skin(SK)	SKE	127	61.65	6	45.61	113	54.85	6	9.83
	SKA	79	38.34	53	54.38	93	45.14	53	86.88
	SKB	0	0	1	0	0	0	1	1.63
	SKNA	0	0	1	0	0	0	1	1.63
Skin Mucus(SM)	SME	60	29.12	13	8.77	99	48.05	13	21.66
	SMA	146	70.87	46	82.45	82	39.8	46	75.4
	SMB	0	0	1	8.77	25	12.13	1	1.63
	SMNA	0	0	1	0	0	0	1	1.63
Eyes(E)	EE	96	46.6	8	29.82	95	46.11	8	13.11
	EA	91	43.68	40	63.15	98	47.57	40	65.57
	EB	19	9.22	13	7.01	13	6.31	13	21.31
	ENA	0	0	0	0	0	0	0	0
Gills(G)	GE	154	74.75	9	78.94	146	70.87	9	14.75
	GA	48	23.3	21	19.29	46	22.33	21	34.42
	GB	4	1.94	30	1.75	12	5.82	30	49.18
	GNA	0	0	1	0	2	0.97	1	1.63
Flesh(F)	FE	195	94.66	29	94.73	158	76.69	29	47.54
	FA	11	5.33	3	5.26	43	20.87	29	47.54
	FB	0	0	0	0	5	2.42	3	4.91
	FNA	0	0	0	0	0	0	0	0

SK: Skin, **SKE**: Bright, iridescent pigment, opalescent, no discoloration, **SKA**: Pigmentation bright but not lustrous, **SKB**: Pigmentation in the process of becoming discolored and dull and **SKNA**: Dull pigmentation, **SM**: Skin mucus, **SME**: Aqueous, transparent, **SMA**: Slightly cloudy and **SMB**:Milky, **SMNA:**Yellowish, grey, opaque mucus, **E**: Eyes, **EE**: Convex, black, bright pupil, transparent cornea, **EA**: Convex and slightly sunken, black, dull pupil, **EB**: Flat, opalescent cornea, opaque pupil and **ENA**: Concave in the center, grey pupil, milky cornea, **G**: Gills, **GE**: Bright color, no mucus, **GA**: Less colored, transparent mucus and **GB**: Brown becoming discolored, thick opaque mucus, **F:** Flesh, **FE:** Firm and elastic, smooth surface, **FA:** Less elastic, **FB:** Slightly soft, less elastic and **FNA:** Soft, scale easily detached from skin, surface rather wrinkled

Table: 2 Quality Index(QIM) score for fishes [Indian major carp] at Shop and road side vendorsP

Quality parameter		Description	G_S	Shop		Road vendors	
				S_I	S_E	RV_I	RV_E
Skin	Color/ Appearance	Pearl-shiny all over the skin	0	60.19	47.36	55.82	9.83
		Skin is less pearl-shiny	1	39.8	52.63	44.17	86.88
		Fish is yellowish, mainly near abdomen	2	0	0	0	3.27
	Mucus	Clear, not clotted	0	31.06	10.52	68.93	26.22
		Milky, clotted	1	68.93	80.7	31.06	72.13
		Yellow and clotted	2	0	8.77	0	1.63
	Odor	Fresh sea weedy, neutral	0	100	100	98.54	63.93
		Cucumber,metal,hay	1	0	0	1.45	39.06
		Sour, dish cloth	2	0	0	0	0
		Rotten	3	0	0	0	0
	Texture	In rigor	0	99.51	100	93.2	78.68
		Finger mark disappears rapidly	1	0.48	0	5.82	16.39
		Finger leaves mark over 3 seconds	2	0	0	0.97	4.91
Eyes	Pupils	Clear and black, metal shiny	0	45.63	26.31	48.54	14.75
		Dark grey	1	44.66	64.91	31.06	67.21
		Matt grey	2	9.7	8.77	5.82	18.03
	Form	Convex	0	86.89	87.71	93.68	52.45
		Flat	1	12.62	12.28	5.33	47.54
		Sunken	2	0.48	0	0.97	0
Gill	Color	Red/dark brown	0	75.72	85.96	74.75	31.14
		Pale red, Pink/light brown	1	22.33	14.03	20.87	27.86
		Grey-Brown, Brown, Grey, Green	2	1.94	0	4.36	40.98
	Mucus	Transparent	0	29.61	7.01	39.32	13.11
		Milky, clotted	1	37.37	82.45	53.88	45.9
		Brown, clotted	2	7.76	10.52	6.79	40.98
	Odor	Fresh, seaweed	0	98.54	100	94.66	63.93
		Metal, cucumber	1	1.45	0	4.36	34.42
		Sour,mouldy	2	0	0	0.97	1.63
		Rotten	3	0	0	0	0
Flesh	Texture	Firm	0	93.2	94.73	77.18	49.18
		Rather soft	1	6.79	5.26	21.35	45.9
		Very soft	2	0	0	1.45	4.91
	Color	White, grayish	0	99.02	100	96.11	85.24
		Some yellowish, little pinkish	1	0.97	0	3.88	14.75
		yellow, over all pink	2	0	0	0	0
Quality Index			0-24	3	3.58	2.86	7.26

GS: General Score**, SI:** Shop Initial**, SE:** Shop End

RV_I: Road vendors initial, **RV_E:** Road vendors end

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RESEARCH ARTICLE

Documentation of Zooplankton in Fish Seed Rearing Ponds of M.N Camp Farm, Chikmagalur District, Karnataka

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ABSTRACT

The present study deals with the documentation of zooplankton composition in fish seed culture ponds at M.N camp of Karnataka. During this study, 25 species of zooplankton were recorded of which rotifera and cladocerans are represented by 9 species followed by copepod with 03 species. While, Ostracoda and protozoans are represented by 02 species each. Total zooplankton groups were in the order of Rotifera > Copepoda > Cladocera > Ostracoda > Protozoa. This findings revealed that the surface quality of the ponds are productive in nature as per zooplankton composition. Fish ponds of M.N camp farm are used to rear and produce fish seeds of common carp and Indian major carps fishes and zooplankton are the main food for these fishes. Hence, the ponds should be appropriately maintained for the future generation.

Key Words: Fish seed rearing, Ponds, M.N camp, Chikmagalur district, Zooplankton, Groups

INTRODUCTION

Ponds are the used by man for various purposes. However, there is a close relation between the metabolism of aquatic organism and hydrobiological characters in a freshwater habitat (Deshmukh & Ambore, 2006; Tapas Kumar Dutta and Bidhan Patra, 2013). Ponds provide habitat for aquatic organisms which play a role in maintaining biodiversity. Zooplankton are drifting in water especially in pelagic and littoral zones in lentic and lotic freshwater habitat (Fernanado *et.al.,* 1990; Shashank and Raghunandan, 2020).

The demands on freshwater habitats have risen by the human beings over the last century leads to large and threats of biodiversity around the world (Dudgeon *et.al.,* 2006; Sivakami *et.al.,* 2015). Globally, documentation of depletion of biological diversity, identifying their causes and finding solutions have a major part of freshwater ecology (Strayer and Dudgeon, 2010). In many lakes and reservoirs zooplankton population have been shown variations in abundance of specific taxa in the late spring through pre-monsoon especially in the tropics. This seasonal pattern in zooplankton are driven by a combination of abiotic factors (Moore and Folt, 1993; Sivakami *et.al.,* 2015; Benndorf *et.al.,*2001), nutrients (Urabe *et.al.,* 1997) as well as biotic factors (Gliwiez & Pijanowska, 1989; Sivakami *et.al.,*2015). Hence, the current study was undertaken to know the zooplankton community in fish seed rearing ponds of M.N. Camp, Chikmagalur District, Karnataka.

Zooplankton play a major significant role in the production of energy and circulation throughout food web in the water bodies. The diversity, density and distribution of zooplankton are influenced by environmental factors in which they live (Sonic Patritia and Martin, 2017). Zooplankton are cosmopolitan in freshwater bodies. Their composition and abundance are of eco importance, as they are bioindicator sensitive organisms (Shashank and Raghunandan, 2020). Keeping this in view the present study is undertaken to document the zooplankton composition in fish culture ponds of M.N camp fish seed farm, Karnataka

MATERIALS AND METHODS

Study Area

M.N Fish seed rearing centre is under the management of Training center of Bhadra fish farm, Karnataka which is situated at 13º 41' N latitude and 75º 38' E longitude. The area of this farm is about 35 acre with 72 earthen ponds and 02 cement cistern ponds for fish seed rearing. The depth of these ponds varied from 2-5 meters. Few ponds are completely earthen and some are side cement revitted bottom earthen ponds.

Zooplankton Analysis

Zooplankton samples were collected on monthly basis from August 2012 to July 2013. The plankton net is formed of bolting nylon silk (mesh- size 50µm) is employed for collection and which is conical in shape and reducing cone with the bottle at its end. For a particular collection , the plankton net is towed horizontally and obliquely (for Qualitative) in surface water. About 100 liters of water is filtered through plankton net. Zooplankton samples were washed in bottles and preserved by adding 5% formalein solution. 1 ml of the preserved sample was put on a Sedgwick-Rafter counter cell and observed it under a microscope. Theyare identified as per the standard keys (Edmondson, 1959; Needham and Needham, 1962; Pennak, 1978;Tonapi, 1980) and results were expressed as Organisms per liter (O/L).

Statistical Analysis

One-way ANOVA and Post Hoc Tukey HSD test for zooplankton samples are designed to compare the means of three or more independent samples simultaneously by using socscistatistics software.

RESULTS AND DISCUSSION

Table 3 depicts the occurrence of zooplankton groups in fish seed rearing ponds of M.N. Camp, Chikmagalur district, Karnataka. However, Table 4 shows total zooplankton populations. Seasonal fluctuations (%) of zooplankton in fish seed rearing ponds are depicted in Table 5 and Figure 3. While, Table 6 data showed seasonal variations of zooplankton diversity indices. Table 7 depicts regression seasonal data for abundance of zooplankton belongs to different groups. Figure 2 highlights the percentage of zooplankton groups in fish seed rearing ponds.

The protozoan population ranged from 90 o/l (September) to 670 o/l (June). Seasonally, they are maximum in rainy season with 12% and minimum during winter season (7%). However, total Rotifera population fluctuated between 440 o/l (July) and 5100 o/l (February) (Table 4). Season wise, rotifers are highest in summer (30%) and lowest in rainy season with 21% respectively (Table 5). The density of copepods deviated from 60 o/l (February) to 700 o/l (October). They are maximum in rainy season (29%) and minimum in summer season with 20% respectively. Lowest population of cladocerans were recorded in the month of July with 40 o/l and highest cladocerans were noticed in the month of April with 1300 o/l. Their population was highest in rainy season (25%) and lowest during summer and winter seasons (24%). Ostacods were maximum with 360 o/l in the month of May and minimum in the month of July with 36 o/l respectively. Seasonally, ostracods were highest in winter season (18%) and lowest in rainy season with 12% respectively. If the total zooplankton groups were taken into consideration rotifers were dominant with 25.33% followed by copepod (25%), Cladocera (24.33%), Ostracoda (15.67%) and Protozoa (9.67%).
Rotifera > Copepoda > Cladocera > Ostracoda > Protozoa.

Seasonal regression data for protozoans were highest in summer vs. rainy season and lowest during winter vs. rainy season. Rotifers were maximum in summer vs. rainy and minimum during winter vs. rainy season. Regression data is maximum in winter vs. summer season and minimum in winter vs. rainy season for copepods. Similarly for Cladocerans regression values were high during winter vs. rainy season and low during summer vs. rainy season. However, for Ostracods the data is maximum during winter vs. summer and minimum in summer vs. rainy season (Table 7). Species spectrum of zooplankton is depicted in Table 3. Rotifers and Cladocera were the dominant groups with 9 species followed by Copepoda (3 species). While, Ostracoda and protozoans are represented by 02 species each respectively.

Shannon Weiner diversity index of species is maximum during summer and minimum during rainy season. Species richness is highest in summer and lowest in winter season (Table 6). Vipul Sharma et al., (2012) opined that nutrients, weeds and depth of the water bodies favored rich abundance of cladocerans. Cladocerans are food source for fry, fingerlins and adult of the many food fishes. Cladocerans are the indicators of eutrophic water bodies (Sharma, 2001). Harish Kumar and Kiran (2020) reported 06 species of cladocerans belonging to 6 families and 07 genera and 07 species of copepods from sewage fed tank of Bhadravathi taluk, Karnataka. They observed best density of copepods during September and their number was least in May. They observed that nutrients like sulphate, nitrate, phosphate contents were high during December and January which harbor good number of Cladocerans. Their findings revealed that the surface quality of the water body is productive with nutrient rich status.

Bharati et al., (2014) reported that the abundance of Rotifera in nutrient rich water body which undergo the state of eutrophication. Globally, rotifers have 500 species of which 330 species belonging to 63 genera and 25 families have been authenticated and were described from Indian water bodies (Arora and Mehra, 2003; Kiran et al., 2007; Tanmay Datta, 2011). Harish Kumar and Kiran (2015) have assessed the rotifers in Jannapura tank near Bhadravathi town of Shivamogga district . Total of 05 genera, 08 species of Rotifera belonged to 4 families were recorded by them. The family Brachionidae consists of 5 species and rest of the families consists of single species of rotifers respectively.

One-Way ANOVA and Tukey HSD test (Table 1)

Post Hoc Tukey HSD

The Tukey's HSD (honestly significant difference) procedure facilitates pair wise comparisons within ANOVA data. The F statistic (above) tells whether there is an overall difference between sample means. Tukey's HSD test allows to determine between which of the various pairs of means - if any of them - there is a significant difference. A couple of things to note. First, a blue value for Q (below) indicates a significant result. Second, it's worth bearing in mind that

there is some disagreement about whether Tukey's HSD is appropriate if the F-ratio score has not reached significance.

CONCLUSION

Based on the zooplankton of fish seed rearing ponds of M.N camp the water is suitable for fish culture. The species such as *Brachionus, Keratella, Fillina*, indicate organic pollution as an account of disposal of cow dung and poultry waste. Fish ponds of M.N camp fish farm are used to rear and produce fish seeds of *Cyprinus carpio* and Indian major carps (Catla, Rohu, Mrigal and zooplankton are the main food for these fishes. Hence, it becomes necessary that the ponds should be appropriately maintained for the future generation.

REFERENCES

1. Arora J, Mehra N K.2003.Seasonal diversity of planktonic and epiphytic rotifers in the backwaters of the Delhi segment of the Yamuna River, with remarks on new records from India. Zoological Studies; 42:239-249.
2. Bharati G, Kolhe Shinde S M, Rane M S.2014. Composition and Biodiversity of Rotifer Population in Godavari River. Indian Journal of Applied Research Volume 4(6):554-556.
3. Benndorf, J., Kranich, J., Mehner, T. and Wagner, A. 2001. Temperature impact on the midsummer decline of Daphnia galeata: an analysis of long-term data from the biomanipulated Bautzen reservoir (Germany). Freshwater. Biol. 46: 199-211.
4. Deshmukh, J.U. and Ambore, N.E. 2006. Seasonal Variations in Physical Aspects of Pollution in Godavari River at Nanded, Maharashtra, India. Journal of Aquatic Biology 21(2): 93-96.
5. Dudgeon, David & Arthington, Angela & Gessner, Mark & Kawabata, Zen-Ichiro & Knowler, D. & Lévêque, Christian & Naiman, Robert & Prieur-Richard, Anne-Hélène & Soto, D. & Stiassny, Melanie & Sullivan, Caroline. 2006. Freshwater Biodiversity: Importance, Threats, Status and Conservation Challenges. Biological reviews of the Cambridge Philosophical Society. 81. 163-82.
6. Edmondson, WT (1959). Freshwater Biology (2nd edition). John Wiley & Sons, New York, 1248 8..
7. Fernando, C.H. Tudorancea, C, Mengestou, S. 1990. Invertebrate Zooplankton Predator Composition and Diversity in Tropical Lentic Waters. In: Intra Zooplankton Predation Springer, Dordrecht: 13-31.
8. Gliwicz, Z. M. and Pijanowska, J. 1989. The role of predation in zooplankton succession. In: Sommer, U. (ed.), Plankton Ecology: Succession in Plankton Communities. Springer- Verlag, New York, pp. 253-295.
9. Harish Kumar, K and Kiran, B.R. 2015. Population dynamics of Rotifers in Jannapura tank, Karnataka. International Journal of Fisheries and Aquatic Studies 2015; 3(1): 165-168
10. Harish Kumar, K and Kiran, B.R. 2020. Documentation on distribution and abundance of Micro-Crustaceans (Cladocera and Copepoda) in Sewage Fed tank of Bhadravathi taluk, Karnataka. CIBTech Journal of Zoology Vol.9:.107-114.
11. Kiran BR, Puttaiah ET, Kamath D.2007. Diversity and seasonal fluctuation of zooplankton in fish pond of Bhadra fish farm, Karnataka. Zoos, Print Journal.22:2935- 2936.
12. Moore, M. and Folt, C. 1993. Zooplankton Body Size and Community Structure: Effects of Thermal and Toxicant Stress. Trends in Ecology and Evolution 8: 178-183.
13. Needham JG and Needham PR (1962). A Guide to the Study of the Freshwater Biology. Holden-Dey Inc., San Francisco, 108 pp.
14. Pennak RW(1978). Freshwater Invertebrates of United States. 2nd ed., John Wiley & Sons Inc., New York, 803pp.
15. Sharma BK. 2001. Biological monitoring of freshwaters with reference to role of freshwater Rotifera as biomonitors. In: Water Quality Assessment Biomonitoring and Zooplanktonic Diversity (B.K.Sharma).Ministry of Environment and Forests, Government of India, New Delhi: 83-97.

16. Shashank, K.R. and Raghunandan, K.S. 2020. A checklist of Zooplanktons recorded at Melukote ponds, Pandavapura taluk, Karnataka. International Journal of Fisheries & Aquatic Studies 8(3): 268-272.
17. Sivakami, R, Arumugam, V. and Premkishore, G. 2015. An analysis of Zooplankton in a lake, Pudukkotai district, Tamilnadu, India. International Journal of Current Microbiology and Applied Sciences 4(5): 377-389.
18. Sonic Patritia, A and Martin, P. 2017. A Survey on seasonal variation of freshwater zooplankton diversity in Kolavai lake, Chengalpattu, Tamilnadu, India. World Journal of Pharmaceutical Research Vol. 6(10): 1732-1744.
19. Strayer David L. and David Dudgeon.2010. Freshwater biodiversity conservation: recent progress and future challenges. Journal of the North American Benthological Society Vol. 29, No. 1 :344-358.
20. Tanmay Datta.2011. Zooplankton diversity and physicochemical conditions of two wetlands of Jalpaiguri district, India. International Journal of Applied Biology and Pharmaceutical technology issue. 2(3):576-583.
21. Tapas Kumar Dutta and Bidhan C. Patra, 2013. Biodiversity and seasonal abundance of zooplankton and its relation to physico-chemical parameters of Jamunabundh Bishnupur, India. International Journal of Scientific & Research Publications Vol. 3(8): 1-7.
22. Tonapi GT(1980). Freshwater Animals of India: An Ecological Approach. Oxford and IBH Publishing Co., New Delhi, India. p.341.
23. Urabe, J., Clasen, J. and Sterner, R. W. 1997. Phosphorus limitation of Daphnia growth: Is it real? Limnol. Oceanogr., 42: 1436-1443.
24. Vipul Sharma, Bhoopendra Kumar Verma, Ridhhi Sharma, Madhu Sudan Sharma, Kuldeep Singh Gaur (2012). A report on the freshwater Cladocera (Crustacea: Branchiopoda) of south Rajasthan (India). International journal of Environmental Sciences, 3(1) 275-296.

Table 1. One-Way ANOVA and Tukey HSD test

	Protozoa (T1)	Rotifera (T2)	Copepoda (T3)	Cladocera (T4)	Ostracoda (T5)	Total
N	12	12	12	12	12	60
∑X	4080	28870	3380	5460	1656	43446
Mean	340	2405.8333	281.6667	455	138	724.1
$\sum X^2$	1877400	92611500	1513000	4570600	343546	100916046
Std.Dev.	211.101	1450.8647	225.8251	435.5039	102.2555	1085.0041

Source	SS	df	MS	
Between-groups	43049221.0667	4	10762305.2667	*F* = 22.41504
Within-groups	26407576.3333	55	480137.7515	
Total	69456797.4	59		

The f-ratio value is 22.41504. The p-value is < .00001. The result is significant at p < .05

Table 2. Post Hoc Tukey HSD

Pair wise Comparisons		$HSD_{.05}$ = 797.8145 $HSD_{.01}$ = 967.7388	$Q_{.05}$ = 3.9885 $Q_{.01}$ = 4.8380
T_1:T_2	M_1 = 340.00 M_2 = 2405.83	2065.83	Q = 10.33 (p = .00000)
T_1:T_3	M_1 = 340.00 M_3 = 281.67	58.33	Q = 0.29 (p = .99958)
T_1:T_4	M_1 = 340.00 M_4 = 455.00	115.00	Q = 0.57 (p = .99409)
T_1:T_5	M_1 = 340.00 M_5 = 138.00	202.00	Q = 1.01 (p = .95241)
T_2:T_3	M_2 = 2405.83 M_3 = 281.67	2124.17	Q = 10.62 (p = .00000)
T_2:T_4	M_2 = 2405.83 M_4 = 455.00	1950.83	Q = 9.75 (p = .00000)
T_2:T_5	M_2 = 2405.83 M_5 = 138.00	2267.83	Q = 11.34 (p = .00000)
T_3:T_4	M_3 = 281.67 M_4 = 455.00	173.33	Q = 0.87 (p = .97248)
T_3:T_5	M_3 = 281.67 M_5 = 138.00	143.67	Q = 0.72 (p = .98625)
T_4:T_5	M_4 = 455.00 M_5 = 138.00	317.00	Q = 1.58 (p = .79501)

Table 3. Occurrence of Zooplankton groups in fish seed rearing ponds of MN Camp, Chikmagalur district, Karnataka

Rotifera	Copepoda	Cladocera	Ostracoda	Protozoa
Brachionus falcatus *Brachionus plicatilis* *Brachionus caudatas* *Filinia longiseta* *Keratella tropica* *Rotaria sp.* *Monostyla bulla* *Brachionus angularis* *Brachionus rubens*	*Mesocyclops hyalinus* *Nauplius larvae* *Cyclopoid naupli*	*Moinadaphnia* *Daphnia carinata* *Diaphanosomasarsi* *Cypris sp.* *Bosmina longirostris* *Ceriodaphnia sp.* *Moina sp.* *Scapholeberis sp.* *Macrothrix sp.*	*Cypris subglobosa* *Heterocypris*	*Arcella vulgaris* *Diffugia sp.*

Table 4. Total Zooplankton population of fish seed rearing ponds of MN Camp, Chikmagalur district, Karnataka

Sl. No.	Zooplankton groups	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July
1	Protozoa	150	90	110	140	240	300	350	480	600	640	670	310
2	Rotifer	980	2200	1950	1650	2500	3800	5100	4500	3000	1800	950	440
3	Copepod	450	680	700	260	140	130	60	80	100	160	220	400
4	Cladocera	60	50	100	210	320	580	800	1100	1300	700	200	40
5	Ostracoda	55	170	120	60	40	65	80	200	240	360	230	36

Table 5. Seasonal fluctuations (%) of Zooplankton in fish seed rearing ponds of MN Camp, Chikmagalur district, Karnataka

Season	Protozoa	Rotifera	Copepoda	Cladocera	ostracoda
Winter	7.0	25	26	24.0	18
Summer	10.0	30	20	24	17
Rainy	12.00	21	29	25	12
Total	9.67	25.33	25.0	24.33	15.67

Table 6. Seasonal variations of Zooplankton diversity indices of fish seed rearing ponds of MN Camp, Chikmagalur district, Karnataka

Sl. No.	Season	Shannon Weiner diversity index of species (H')	Shannon Weiner index for family (H)	Species richness (d)
1	Winter	1.70	0.95	0.90
2	Summer	1.90	1.30	1.70
3	Rainy	1.62	1.10	1.60

Table 7. Regression seasonal data for abundance of Zooplankton belongs to different groups in fish seed rearing ponds of MN Camp, Chikmagalur district, Karnataka

Seasons	Protozoa	Rotifer	Copepoda	Cladocera	Ostracoda
Summer vs. Rainy	0.545	0.560	0.635	0.102	0.000
Winter vs. Rainy	0.207	0.058	0.10	0.485	0.364
Winter vs. Summer	0.490	0.525	0.80	0.375	0.410

Figure 1: Fish pond of M.N camp used for Zooplankton sampling

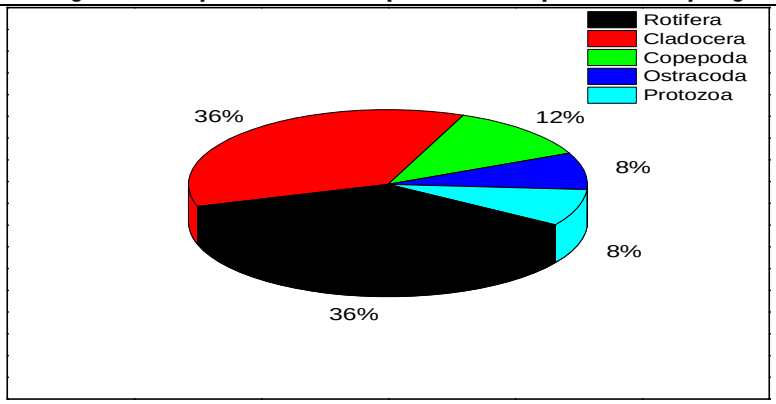

Figure 2: Percentage of zooplankton groups in fish seed rearing ponds of MN Camp, Chikmagalur district, Karnataka

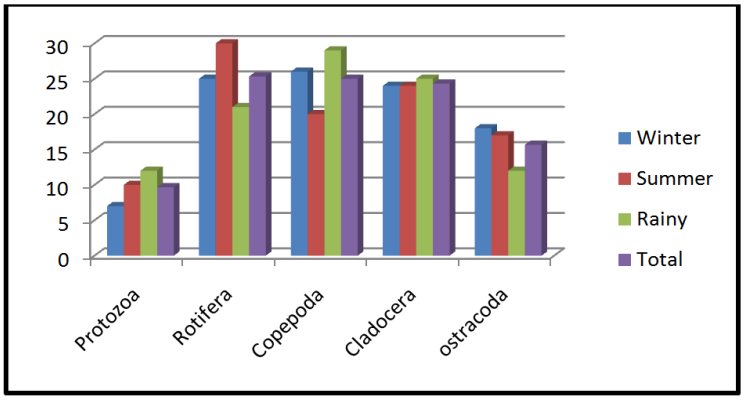

Figure 3: Seasonal fluctuations (%) of Zooplankton in fish seed rearing ponds of MN Camp, Chikmagalur district, Karnataka

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RESEARCH ARTICLE

Sentiment Analysis of Twitter Data

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ABSTRACT

Sentiment Analysis is the methodology to study the individual opinions of user on any subject like appraisal or expressing an emotion, product review etc. In this method we are using the Lexicon based approach, where an external dictionary is used for comparison. The phrases or tweets are classified primarily into two classes - Positive and Negative. The tweet sentiment polarity is extracted, and the score is calculated and generated visually. This score helps is understanding whether the given set is positive or negative sentences.

Keywords: Twitter data, Analysis of Sentimence, Lexicon-based approach

INTRODUCTION

In the present world people use technology and social sites more and as a result data is generated in bulk (big data). A methodology came into existence to analyse these reviews known as Sentiment Analysis- the process of identifying the emotion of the phrases and thereby categorising it as negative, positive, neutral etc and thus helps in important decision-making processes. Any word in this world has an emotion. The immense possibilities of analysing the sentimence came with market value after the boom of Artificial Intelligence. We cannot make an argument on which is better as a human analysis or a machine prediction, but human race can be proud of the artificial intelligence we make.

Literature Review

One of the first and most popular work in the field of Twitter sentiment analysis was done by Go et al(2009). This research covers up the disadvantage of manual annotation by introducing distant supervision technique. “.)” – Tweets marked with this emoticon is considered as positive and “.(“- tweets with this emoticon were considered as negative. Another popular work was conducted by Pak and Paroubek (2010). Here the distant procedure was used,

and tweets are generalized into negative, positive based on emoticons it mentioned. Addition to this, neutral emotions were considered to show the need of its significance as well. Opinion mining can be described as the self – administered content investigation and rundowns of things accessible on networks that understands our opinions and analyse as positive and negative to know the feelings.

Use of Sentiment Analysis

Politics: Used topredict Election result, which is the most popular usage. Addition to this it monitors the political view – mainly to identify the reliability between statements at any level of administration.
Business and Marketing: Organizations utilize it to create strategies and plans, to analyse the sentiment of users towards items, products or brands and even analyse the future of the business thus.

PROPOSED METHODOLOGY

Collection of Datasets

Twitter Data acts as the source dataset for our research and study of analysis here. It collects the tweets and studies it and analyse with the methodologies mentioned further. At first the twitter account is registered. Once the connection is made application need to be registered on Twitter API. We are provided with four legal credentials for the connection establishment and with the help of **RoAuth** package the connection is established to get the dataset. We retrieve data live here. The legal credentials required for the proposed analysis for twitter data are:

- API (Application Programming Interface) Key
- API (Application Programming Interface) Secret Key
- Access Token
- Access Token Secret

Noise Removal from Tweets

Any dataset must be clean enough to increase its performance thereby we remove any type of noise present in it. The key factor for noise removal is that while scanning any datasets, the useless data is scanned which consumes lot of CPU time which is a wastage as it doesn't provide any meaning in analysing the sentiment of the data.
The noise categories include – The emoticons, URLs, Hashtag, Target

Emoticons: We are much familiar with the emoji's, the pictorially represented facial expressions. The attitude of the user is being expressed here. Though it plays main role in emotion we remove it for not creating confusion to the algorithm
URL: User at times attach the hyperlinks to connect to a particular web address.
Hashtag: Twitter is much famous for hashtags and users use to mark subjects with it.
Target: The @ symbol is usually used to target the user and alert them. It only enhances the visibility of tweet and not play any role in sentiment detection.

Lexical Analysis

In one-word Lexical Analysis can be termed as -By comparing uni-grams to the pre-loaded word database, the tweet is assigned sentiment score - positive, negative, or neutral and overall score is calculated. Tweets are divided into words called as **lexemes**. This is being compared with the dictionary word set. The positive and negative words which are already created acts as the dictionary for the process of Lexical analysis. Based upon user the way of expressing their opinion changes. For example: "This looks so good" is the normal usage of an emotion, whereas people express it in this way too "This luks so gud". This is too considered in the dictionary dataset. The usage of Hybrid language, i.e., combination of multiple language is a challenge for analysis.

For example, "Jobiho hum happy hei". These set of hybrid language is not considered for sentiment analysis as the usage and resources for analysing these sets are low in availability.

Calculation of Score

While in Human validation, the phrases are tagged with the help of guidelines of analysing the right emotion. here tweets are tagged and classified as positive or negative. The classification of words is done by finalising the scores result we get after analysis. Words are checked and matched. Every positive word has a +1 word score and every negative word has a -1 word score. The neutral emotion will be given a score 0.

The formula for calculating "score" is given below
Score = Pos(x, "P word") – Neg(x, "N word")
where, x is a phrase, P word is positive words and N word is negative words

Here each lexeme is compared with the dictionary and thus score is assigned to determine the polarity. In text 1, we find the word "assault" which is a negative emotion and hence the negative score is added here creating a Neg Percent as well. In text 2, we don't find any lexemes with negative or positive word set making it having no score. Thus, it ends as a neutral emotion.

EXPERIMENTAL RESULT

In this segment, we conclude the output obtained after each phase of analysis. For the analysis we concentrate for small range of tweets. If we consider parameters like Hybrid language and large dataset range the process becomes complex. It will be worked on future with new techniques.

Percentage Calculation

In the table tab we have score, tweets, Positive and Negative percentage polarity in the text.
The procedure is by simple arithmetic which helps in easy and better understanding.

Top Trending Tweet Tab Today

A unique feature that displays the top trending hashtags on Twitter based on the geographic location. The feature known as WOEID (Where On Earth Identifier) is used for identifying trending tweets based on location which is a unique 32- bit reference identifier.
R uses WOEID to obtain the trending hashtags from the location

Word Cloud and Histogram

The most useful visual representation of text data, mainly for the metadata is Word cloud. This helps in quickly perceiving most repeatedly used words and for locating a term alphabetically to determine its relative prominence. The packages tm and word cloud are used. The words combined with hashtag is pictorially represented. A histogram plot analyses the intensity of emotions in tweeters and represent the positive, negative, and Overall scores.

Top Tweeters of Hashtag Tab

The Top tweeters of the given hashtag is represented as a bar plot based upon the frequency of tweets. The last 7 days of tweets are considered here. A table with Username of the tweeter and the frequency of tweet gives a better analysis and understanding.

CONCLUSION

A good decision is always appreciated. Similarly, a good understanding and analysis of the words and it's sentimence is needed for improvement in any field. We have two major approaches for sentiment analysis- Machine learning approach and Lexical Analysis. Here we have used a Lexicon based approach. The dataset of twitter is analysed with the powerful set of dictionaries- (negative and positive word sets). R is one amongst the best tool to be

used in sentiment analysis. The score of positive word and negative word is calculated and gives the polarity. Based upon the overall score we can conclude that whether the sentence belongs to positive or negative words. For future improvement, we would be using the Hybrid languages, Emoticons, Slang words (sarcastic emotions), discourse words etc along with the machine learning approach for an advanced experience.

REFERENCES

1. Devika MD, Sunitha C & Ganesh A, "Sentimental Analysis: A comparative Study on Different Approaches", procedia computer Science, Vol.87, (2016), pp.44-49.
2. Jandail RRS, Sharma P & Agrawal C, "A Survey on Sentiment Analysis and Opinion Mining: A need for an Organization and Requirement of a Customer", IJETAE, Vol.4, No.3,(2014), pp.17- 24.
3. Akshi Kumar and Teeja Mary Sebastian, "Sentiment Analysis on Twitter", International Journal of Computer Science Issues, Volume 9, Issue 4, No. 3, July 2012
4. Pierre Ficamos, Yan Liu, "A Topic based Approach for Sentiment Analysis on Twitter Data", International Journal of Advanced Computer Science and Applications, Vol. 7, No. 12, 2016.
5. Pooja Khanna, Sachin Kumar, Sumita Mishra, Anant Sinha, "Sentiment analysis: An approach to opinion mining from twitter data using R", International Journal of Advanced Research in Computer Science, Volume 8, No. 8, 2017
6. H. Thakkar and D. Patel, "Approaches for sentiment analysis on twitter: A stateof-art study," arXiv preprint arXiv:1512.01043, 2013.

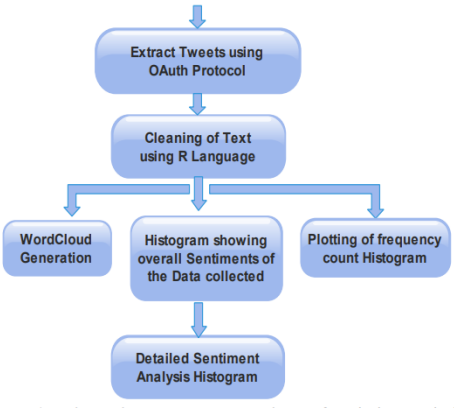

Figure (a). Proposed Methodology

Text	Positive	Negative	Score	PosPercent	NegPercent
Subtitles . Goodra , Nomadic Traders , Assault Industries , Still State . BTW #Modi ji was doing some go /9qvHaK0Tey	0	1	-1	0.00	100.00
PM Modi reviews Covid situation, calls for constant genome sequencing #Modi #Covid #genomesequencing #YesPunjab /6pcPKi0MGh	0	0	0	0.00	0.00

Figure (b). Calculation of Score

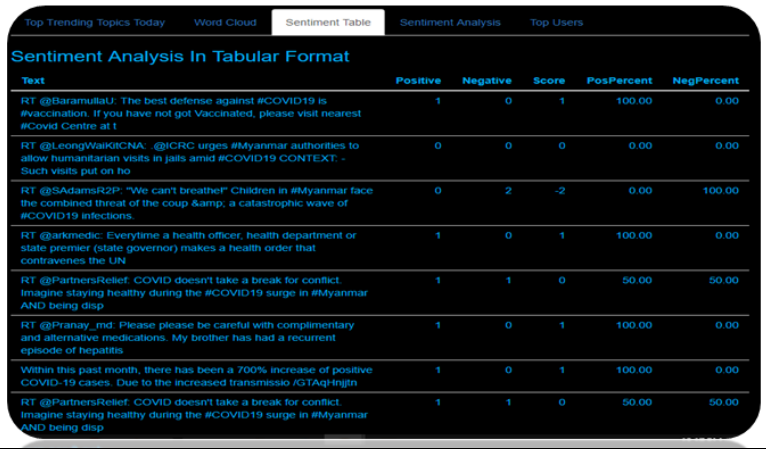

Figure (c). Percentage Calculation

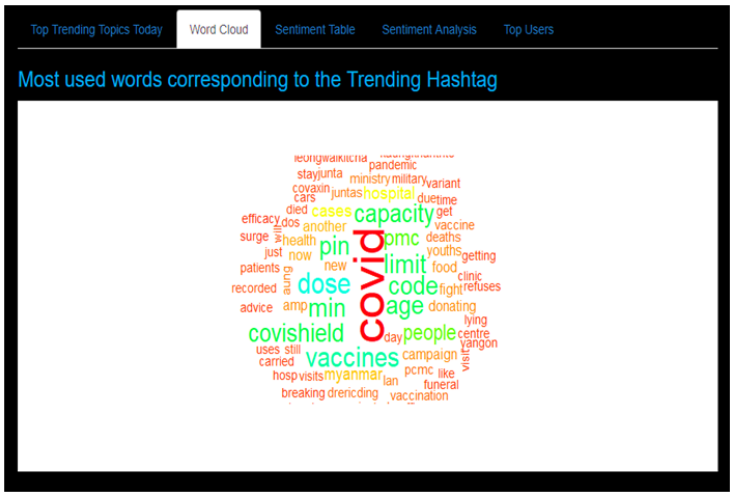

Figure (d). Word cloud and Histogram

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RESEARCH ARTICLE

The Model of Holistic Research System for Vibrant and Vigorous Higher Education Institutions in India

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ABSTRACT

Indian higher education institutions (HEIs) have multiple chronic problems like socio-cultural ecosystem, educational environment, government policies, statutory framework, obsolete curriculum, rigid pedagogy, conventional evaluation, low quality of knowledge and faculty, lack of research support, shortage of budgetary provisions, lack of infrastructure and technological support and so on. Though the higher education is the foundation of policy making and development processes, it could not become the first priority of the nation. The HEIs could not stand as the global players in academia and employment market and also failed to establish strong collaborations with industries and private corporations through research, innovative entrepreneurship and supply of skilled man power. There is lack of integrity, accountability and coordinated efforts, ignorance, indifference, pessimistic approach, status-quo mind-set and stagnancy of results and prospects. These problems are not of funding or physical resources, but pertain to cognitive and intellectual aspects and relate to under-utilisation of available resources in the HEIs. This research paper is a theoretical exploration of a dynamic solution to these chronic problems through holistic research system at the levels of HEIs. The model of 'Holistic Research System' includes Academic Research, Research Education and Researchology, Research System, Systemic Research, Research Outcome, Research Monitoring and Research Assessment. This research also highlights the vital role of educational entrepreneurship and spirit of transformation for obtaining the desired goals within the existing resources and budgets through the holistic research system.

Keywords: chronic problems, higher education institutions, holistic research system, educational entrepreneurship, dynamic solution.

INTRODUCTION

Higher Education Institutions (HEIs) are the cradle of research and the research system is the guardian of HEIs. The HEIs represent universities, colleges and other stand alone, self-sustained and autonomous institutions imparting education at tertiary level in India. The process of globalisation created world-wide competition for these HEIs in academic world and employment market and also opened opportunities of global collaborations and partnerships for the HEIs in India. The proportion of private higher educational institutions (HEIs) increased to a large extent in the new millennium and apparently these private HEIs created competition for public funded HEIs. However, the HEIs could not expand globally and most of the private HIEs adopted the existing structure, system and style of operations. This is a problem of systemic inefficiencies and stagnations accompanied by the socio-cultural ecosystem in the country. In fact, this is not a problem of funding or physical resources, but it is a problem of attitude and approach and its solution lies in igniting spirit and inspirations among the stake-holders in the HEIs.

This research article is a study of chronic and contemporary problems in the HEIs in India and a theoretical exploration of holistic research system as the generic solution to these problems. The holistic research system is suggested as a parallel mechanism to the system of management in profit making industries and private corporations. It is evident from the history that, the motivation, spirit and inspirations made revolutions in the world. The motivation, morale and inspirations have also created their place in theory and practice of management in private industries and corporations. The human organisation in the HEIs also need the same spirit, inspirations and encouragement as the prime source of energy, passion, dedication and determination with integrity, ethics and commitment. The holistic research system is a way to enhance the cognitive qualities among the stakeholders and enlighten themselves with the spirit of common cause and national character through educational entrepreneurship.
It is now recognised that 'research' is the only medicine or treatment to invigorate and strengthen the HIEs in India. However, there is confusion about making it work practically, as the attempts are made to increase quantum of research projects, papers or articles only. Mere increase in number of research students and projects/papers/articles will not be able to make any qualitative change in the present situation without a holistic restructuring of the system in the HEIs and involvement of all the stakeholders collectively in this process of systemic change. The holistic research system explored as way to provide a comprehensive and inclusive solution to overcome this situation within the available resources and budget. This research article also presents a theoretical model of research utility and application for consideration of academic world.

METHODOLOGY

This research article is a theoretical exploration innovative idea based in depth study, field experience and personal observations. The ideas, knowledge and information in this research paper are originally generated through study and practice of research in the field of education and social sciences.

The Problems of Higher Education Institutions

Indian higher education system evolved with a background of monopolistic control by a community of a few people and colonial legacy of British policy of creating clerks and bureaucrats. Even after independence, the old structure and rigid system of higher education prevailed through the obsolete course-curriculum, century old pedagogy and unaltered evaluation system accompanied by the centuries-old apathy, ignorance and indifference among the masses. Actually, these are the chronic problems and also the causes of contemporary problems in the HEIs in India. Presently, the HEIs in India are facing the problems of lack of research support, ignorance of national character, lack of integrity to common cause, indifference towards accountability, status-quo mind-set of stake-holders, stagnancy of results and prospects, lack coordinated efforts, and under-utilised resources and as a result, the HEIs are lagging behind in terms of world-class quality of knowledge, infrastructure, management, curricula, faculty and students.

The Planning Commission's Report (2013, 12th Plan,Vol.2) highlighted that, the higher education is critical for developing a modern economy, a just society and a vibrant polity. It equips young people with skills, relevant for the labour market and the opportunity for social mobility. It prepares all to be responsible citizens who value a democratic and pluralistic society. Thus, the nation creates an intellectual repository of human capital to meet the country's needs and shape its future. However, the HEIs in India are failed to play the key role of nation building due to ignorance and indifference towards common cause and national character. The Report (2013, 12th Plan, Vol.2) also pointed out that the Indian higher education continues to have limited research capacity. Low levels of funding and segregation of the country's R& D institutions from universities and colleges have been responsible for the weak research capacity of Indian universities. It is disappointing to note that even the country's top universities remain largely teaching-focused with limited research and doctoral education. This lack of research orientation, even in the best of the Indian institutions, is reflected in their standing in global rankings, most of them rely heavily on measurable indices of research performance. No Indian University figured amongst the top 200 universities in the Times Higher Education (THE) Rankings or the Academic Ranking of World Universities (ARWU) for the year 2011.

Higher Education Statistics

To understand the problems of higher education in India, we have to go through the figures of All India Survey of Higher Education (AISHE-2018-19). According to AISHE-2018-19, the Gross Enrolment Ratio (GER) for higher education is 26.3% in 2018 with 37.4 million students. National Education Policy (NEP-2020) of India has set out the target for 2030 as the Gross Enrolment Ratio in higher education shall increase from 26.3% (2018) to 50%. This shows the present and contemplated volume of higher education in India.

Classification of HEIs in India: According to AISHE (2018-19), there are 993 Universities, 39931 Colleges and 10725 Stand-alone Institutes as on 31st March 2018. Out of total 993 universities, there are Central University (46); Central Open University (1); Institution of National Importance (127); State Public University (371); Institution under State Legislature (5); State Open University (14); State Private University (304); State Private Open University (1); Deemed Government University (34); Deemed University - Government Aided (10) and Deemed Private University (80). Thus, there are 608 (61.24%) government aided and public funded and 385 (38.77%) private - unaided Universities in the country. Among these 993 Universities 394 (39.70%) Universities are located in rural areas.

The Problems of HEIs in India

The HEIs in India are classified on the basis of source of funding, nature of management and statutory control as: government aided or public funded; high class public funded; autonomous or stand- alone, high cost privately managed and unaided. These HEIs are facing different as well as similar problems and challenges described as under.

The Aided or Public Funded HEIs: The peculiar status and problems of government aided or public funded HEIs are listed as**:** (i) management by orders and instructions - only follow the procedures and instructions as official formality; (ii) purpose of work is formal duties and earning livelihood income; (iii) lack of progressive approach; (iv) teaching as formality, lack of improvements, involvement and innovations - passive and indifferent approach of faculty; (v) students are degree seekers, no quest for knowledge and learning, no research attitude; (vi) lack of dynamic governance; (vii) lack of professionalism in operations; (viii) lack of professional ethics; (ix) Indifferent students, teachers, administration and the heads of institutions; (x) inefficiency at all levels; (xi) lack of change , innovation and creativity; (xii) lack of self-discipline in students, teachers, staff and academic heads; (xiii) lack of values; (xiv) lack of work-culture; (xv) lack of educational environment; (xvi) lack cleanliness, hygiene, peaceful atmosphere; (xvii) lack of green premises; (xviii) administration is bound by formal duties and habits; (xix) lack of participative administration; (xx) lack of well-established research system; (xxi) lack of accountability; (xxii) pleasure of stability; (xxiii) static mind-set of human resources; (xxiv) "no problem zone and we are doing our duty" approach of human resources; (xxv) resistance for change; (xxvi) stagnation of prospects; (xxvii) lack of ambition among the

stakeholders and (xxviii) lack of proper utilisation of available resources and budgets; (xxix) influential appointments as heads, faculty and personnel and (xxx) compromise with quality, efficiency and under-utilisation of resources.

High Funded Public HEIs: The groups of Indian Institute of Sciences (IISc), Indian Institute of Technology (IITs), National Institute of Technology (NITs), Indian Institute of Science Education and Research (IISERs), Indian Institute of Management (IIMs) and other institutions of national importance are the high funded and specially recognised HEIs in India. Main features of these institutions are: (a) mainly established in big cities; (b) special budgetary provisions - no problem of funds; (c) world class infrastructure, amenities and facilities; (d) high cost and quality of education and (e) high class of faculty and a limited class of students. The main challenge created by these institutions is of brain-drain of the first quality knowledge and product, as most of the students give up India for their career and development after completion of education in the HEIs. Most of them do not return home land and those who come back are engaged in private employment or high profile jobs of individual interests. Ultimately, these institutions have created a perception that their existence is for creating a limited high class of affluent people who have no bearing on the general field of higher education in the nation.

Autonomous or Stand-alone HEIs: These HEIs are autonomous Colleges or Stand-alone Institutions not affiliated to any University and are funded by the government or sustain through their own fund arrangements through fees and donations. Their management and operations are autonomous in terms of curriculum, pedagogy, fee-structure and awarding certificates and some of them are also empowered to award degrees. These Institutions are managed by special bodies of nominated members constituted for that purpose. These Institutions are limited in number, self-sufficient and competent to manage their affairs independently and do not have any bearing on general HEIs in India.

High Cost Private HEIs: These HEIs are less in number and are concentrated in metros and big cities only. These institutions are mainly business schools and technical or professional institutions established for certain high-income groups only. These are established for profit and charge indiscriminate fees to the students. These institutions provide all physical and academic infrastructures, provide necessary training to students as they are inspired by employability factor only and their course-curriculum is prescribed accordingly. These are isolated institutions which do not have any role in general higher education in India.

The Private Unaided HEIs: These privately managed unaided HEIs are established mainly in rural area and also in towns and cities. These HEIs are large in number and spread over across the country. Most of them adopt the easy ways to attract common students - most popular of them is 'guarantee of jobs' in case of professional HEIs and practice of 'no need to attend' classes in the case of Arts, Science and Commerce courses. Main problems in these HEIs are: (a) deceptive advertisements about claims of quality, employment opportunities, amenities and infrastructural facilities in case of professional programmes; (b) common students are attracted to these false claims ignorantly and without confirmation of reality; (c) lack of qualified, competent and sufficient teachers or instructors; (d) lack of infrastructure, educational environment and work culture; (e) lack of sufficient premises and space for classes, library, study rooms and basic amenities; (f) lack of integrity, professional ethics, green, healthy and hygienic premises; (f) classes are not conducted regularly or even without attending any class, students are allowed to appear for final examinations in case of UG and PG courses in Arts, Commerce and Science streams; (g) lack of progressive attitude for change and improvements in operations. These institutions have created a severe impact on the higher education in India as these are large in numbers and spread over to a large extent with influential enrolment of students.

Common Problems and Challenges: The Indian Higher Education is caught between the public funded or aided HEIs and large-scale private institutions. Problems of these two ends are different upto some extent, however, the impact and long-term implications are same ultimately. The problems and challenges are generated through faulty public policy, insufficient funding, excessive privatisation, lack of research system, lack of realisation of common

cause, lack of national character, lack of coordinated efforts, lack of integrity, ethics and commitment, lack of accountability and self-discipline, lack of educational environment, lack of professionalism and professional ethics and lack of dynamic governance and lack of visionary leadership. Main problem of these HEIs are improper utilisation or under-utilisation of available resources and system of mass copy at the time of examination which is common in rural area, small towns and in majority of the HEIs. It is known from the statistics presented in above paragraphs that, majority enrolment in higher education is in Arts, Humanities, Social Sciences, Commerce, Science at UG and PG level programmes where the problems of irregular classes and system of mass copy is peculiar. There are huge number of faculty in public funded and aided HEIs who receive salaries and allied benefits at par with the class-I HEIs in India, but do not conduct their scheduled lectures regularly and sincerely. The overall result of the situation is stagnant, ineffective and inefficient higher education system in India.

Following are the peculiar and common problems in majority of HEIs in India**:** (i) lack of research environment; (ii) lack of research system; (iii) 'let it go' approach; (iv) notion of stability among stakeholders; (v) 'we are doing our job' style of human resources; (vi) work for earning income is the goal of life; (vii) resistance to change and improvements; (viii) under-utilisation of resources; (ix) burden of increasing number of students; (x) decreasing strength of faculty in proportion with the students; (xi) global academic competition; (xii) changing needs of employment market across the world; (xiii) lack of vibrant policies; (xiv) lack of dynamic plans, strategies and governance; (xv) stagnancy of efficiency, results and prospects; (xvi) status-quo mind-set of the stakeholders; (xvii) lack of accountability at all levels and (xviii) improper or under-utilisation of resources and miss-use of national resources. These problems and challenges are the product of "non-research environment and socio-cultural ecosystem" around the HEIs and their stakeholders. Certainly, these are the problems of stakeholders pertaining to their approach, attitude and perceptions. Therefore, the solution of these problems and challenges lies in spirit, inspirations and enlightenment of the stakeholders at the level of HEIs through cognitive and intellectual endeavour. There are arguments like "what can we do; it is not in our hands; it is problem in government; government must increase budget on higher education; there are statutory problems; there is lot of corruption; and so on" among the stakeholders in HEIs. This type of stagnant and status-quo mentality of stakeholders need proper treatment of creating awareness, igniting spirit of common cause and national character through enlightenment of role and responsibilities, purpose of life and sense of achievement.

The Holistic Research System in HEIs

The management system is the heart and soul of operations and organisations in private industries and corporations. Industrial entrepreneurship and innovative leadership are the driving force of operations and management of these industries and corporations. Then, what is heart and soul of academic and administrative operations of higher education institutions? Its simple answer is "holistic research system." Motivation, morale, self-actualisation, hard work, result oriented approach and professionalism are relevant and significant in HEIs also. What is difference in industries or corporations and higher education institutions (HEIs)? Industries and corporations run their operations with human organisations and achieve the desired goals. HEIs also run their operations with human organisations only. The only difference between these two organisations is that the former has management system and the later has no such effective system like management. Therefore, the 'holistic research system becomes relevant and significant in the case of HEIs. As the management system is the heart and soul of organisations in private industries and corporations, the holistic research system is the heart and soul of efficient and vibrant organisations in the HEIs.
The HEIs are running their operations without any benchmarks of performance or with low standards of performance. The resources (physical and human) are utilised inefficiently, improperly and not to the level of their optimum capacity. There are problems of ignorance of national character, lack of research support, lack of integrity to common cause, indifference towards accountability, lack coordinated efforts, status-quo mind-set, pessimistic approach, and stagnancy of efficiencies, results and prospects among the leadership, management, administrative staff, faculty and students in the HIEs. These are the problems of human element among the stakeholders and the centre of these problems is within the HEIs only. At the level of HEIs, the human element among the stakeholders needs a treatment of awareness, inspirations and enlightenment to realise their role, responsibilities, purpose of life

and obligation towards common cause and national interest. It is a historical fact that, when situation is adverse, resources are scarce and weapons are weak, the inner spirit and inspirations of common goal and national character work as the source of energy and revolutions take place. For HEIs, 'holistic research system' is the lighthouse of operations and source of spirit, inspirations and enlightenment of the stakeholders.

Research is an applied science and hence, only teaching-learning of research and granting of some projects is not enough to achieve the desired goals of the HEIs. The real goal of research is application or utilisation of research knowledge and skills in the respective roles and responsibilities in institutional and social life. The process of application and utilisation of research knowledge nurtures the proper approach and attitude of stake-holders and inculcate the research habits among them which finally result in research character, vibrancy and vigour in the HEIs. The continuous process of holistic research system is essential for successful operations and achievement of the desired goals of HEIs which plays multi-dimensional roles of creating spirit and inspirations for change; enlightenment of purpose of life, common cause and national character among stakeholders and in optimum utilisation of available resources in the HEIs.

This model of Holistic Research System is based on the following assumptions

- The statutory framework, government policies, funding position and central monitoring will remain unchanged.
- There are problems of pessimistic approach, static mind-set, notion of stability, and stagnancy of efficiencies and prospects of HEIs in India.
- There is under-utilisation or inefficient utilisation of available resources in the HEIs without proper standards or benchmarks of performance.
- Motivation, morale, self-actualisation, hard work, result oriented approach and professionalism are relevant and significant for vibrant and vigorous HEIs.
- The spirit and inspirations of personal goals, institutional role, common cause and national character helps to change the mind-set of stakeholders in the HEIs.
- This model helps to achieve the desired goals of HEIs within the available resources and existing budget through proper and optimum utilisation of resources.
- This model relies on cognitive efforts, intellectual endeavours and psychological process of creating willingness and interests among stakeholders in the HEIs.
- This model is based on values of integrity, ethics and commitment of the stakeholders within the HEIs.
- Research in higher education is basically action research which has certain utility and practical applications to create vibrancy and vigour in the HEIs.
- Researchology is a trans-disciplinary discipline of study in the faculty of Social Sciences which has its philosophy, theories and principles.

The problems of HEIs can be overcome through cognitive efforts and intellectual endeavours which would enable and help the HEIs create spirit and inspirations among stakeholders including top leadership, management, administrative, faculty and students within the HEIs. The model of holistic research system is the proper and suitable way to end the situation of stagnancy and saturation in the HEIs in India through educational entrepreneurship and innovative leadership. This will help the stakeholders to realise their role and responsibilities at institutional level and enlighten them with the spirit of purpose of life, common cause and national interest. The Stakeholders would be enlightened and inspired through the continuous process of holistic research system and educational entrepreneurship to achieve the desired goals of the HEIs.

Educational Entrepreneurship in HEIs: The model of 'holistic research system' works through educational entrepreneurship in the HIEs. The holistic research system works as a source of energy for all the stakeholders collectively and individually. This system generates academic entrepreneurship with coordinated efforts of top leadership and faculties and heads of the departments. The coordinated efforts of top leadership and administration generate administrative entrepreneurship. This system is most relevant and significant for the faculties and students in the HEIs. This system generates teaching-learning entrepreneurship with the coordinated efforts of faculties and

students of all disciplines. In the same way, the coordinated efforts of administration and students generate entrepreneurship of discipline (conduct) and office services in the HEIs. Thus, the holistic research system provides spirit, inspirations, motivation and energy for top leadership, academics, faculty, administrative staff and students collectively and individually also. The change happens with vibrancy and vigour in the HEIs and educational entrepreneurship works through the coordinated and combined efforts of these stakeholders as a collective endeavour.

Research in Higher Education: Research is the process of critical thinking, careful observations, logical doubts, rational questions, orderly methods, minute inquiries, empirical evidences, practical testing, seeking truth, systematic studies, rigorous discoveries, consistent interpretations, intelligent conclusions and formulation of theories. Knowledge is systematic enlightenment through the results and conclusions of the research. Research aims at generating knowledge and building theories. Knowledge is theoretical description of science. In fact, science is pure form of knowledge ascertained by research. The cycle of knowledge, research and science leads to innovations, technological development and modernization of human life. Creative thinking, problem identification, pointed observations, empirical evidences and proper analysis helps the researcher to move forward towards the ultimate truth. The science is a quest of newness regarding facts, phenomena or problems through the systematic procedure of research. Research makes science as the pure, fine and clear form of knowledge.

The research in higher education is basically action research undertaken for providing solutions to certain problems. It is inherently trans-disciplinary in nature and it has certain practical utility in real life situations. In the words of Best, J.W. et al. (2018) "Action research is focused on immediate application and not on the development of theory or on generalisation of applications. Its purpose is to improve school practices and at the same time to improve those who try to improve the practices; to combine the research processes, habits of thinking, ability to work harmoniously with others, and professional spirit" Thus, action research is prompted by some applications on the basis of results obtained from research. Research is a non-stop intellectual activity driven by analytical mind. Research is a continuous process of experience, observations, knowledge, enlightenment, analysis, and inference, search of alternatives, conclusions, decisions and actions. Research starts with rebelling i.e. a new way of thinking. Out of way thinking, raising questions and visualizing problems are the fundamental characters of research. Research means realization of a problem and the vision of future course of actions. Research is the third eye which sees the unseen and it is the sixth sense which senses the problems, shortfalls and drawbacks as well as the dormant potentials and strengths. Thus, finding a problem and making it as a subject of investigation is the foundation of whole research process and practice. This task is handled through the leadership qualities of a researcher.

Constituents of Holistic Research System

The holistic research system in the HEIs is constituted by constituents as: (i) Academic Research, (ii) Research Education, (iii) Research System, (iv) Systemic Research, (v) Research Outcome, (vi) Research Monitoring and (vii) Research Evaluation. These all constituents are described in the following paragraphs.

Academic Research in HEIs: Academic research is the conventional research undertaken in the HEIs by individuals or team of faculty, scholars and students as a part of their course-curriculum or career advancement. As mentioned in the preamble of STRIDE (2019), "Systematic research can be instrumental in search of truth, quest for learning and understanding experiences in all domains of human existence to improve quality of life." Further, the scope of academic research is highlighted in STRIDE (2019) as, "Research is an essential component of higher education which helps in search of truth, gain insights for creating new knowledge, imparts excitement and dynamism in educational process and facilitates intellectual growth. A trans-disciplinary approach brings specialists from different areas together with a common purpose of evolving new theories, methodologies and frameworks." As mentioned in the STRIDE (2019) guidelines, the objectives of academic research are listed as: (i) to develop research culture and interest among young faculty, scholars and students to pursue careers and undertake the high quality research that augment and expand their research capacity through competitive selection and training to design research, follow

research ethics, write research grant proposals, effectively execute the research and publish and patent research findings; (ii) to motivate young students and faculty to undertake short-term projects analysing local, regional, national problems of development and acquire scientific writing skills, communication and articulation to disseminate research findings and also in developing select bibliographies constructing indices, data-bases; (iii) to encourage critical reasoning, design thing and constructive enquiry to articulate research questions, objectives, hypothesis and methodology; (iv) to promote research entrepreneurship among young faculty, scholars and students through systematic talent hunt, mentorship programs, ideas, innovations, research presentation competitions, annual research seminars and start-up guidance; (v) to promote and strengthen faculty development and post-doctoral research fellowship programs in all disciplines and also promote academic and research ethics and integrity; (vi) to promote exchange of ideas between HEIs and scientists/experts from national bodies and renowned laboratories and (vii) to develop high impact projects and promulgate trans-disciplinary research in the identified thrust areas which have direct implications for creation of new knowledge, advancement of disciplines along with global leadership and progress of the country."Thus, academic research is undertaken by individual researchers or team of researchers, within institution or with collaboration, in the form of research projects, research articles, research papers, paper presentation and so on. Academic research mainly comprises of: (i) teaching research process by teachers - conceptual and theoretical; (ii) learning research process by students as prescribed in course-curriculum; (iii) undertaking small research projects; (iv) writing research articles and papers to publish in journals or conference/seminar proceedings; preparing posters on certain topics or occasions, writing chapters in books and special issues etc.; (v) paper presentation in conferences, seminars etc.; (vi) project work or dissertation for post graduate research degrees; (vii) undertaking sponsored or grantable research projects. Academic research also includes the action research or applied research or evaluation research or post facto undertaken by faculty or students to investigate certain problems or evaluate certain activities or analysis of certain event.

Research Education in HEIs: There is research-illiteracy in India to a large extent. Generally, the stakeholders of the HEIs including top leadership, academics and administrative personnel are formally educated persons. However, they are illiterate in the area of research, as there is dearth of research education in Indian courses and curriculum of higher education. Therefore, there is need for education and training of research to all the stakeholders in the HEIs. The research is not a rigid framework or readymade course for every role and capacity and hence, the education and training of research should be structured suitably for all the stakeholders in the HEIs. Actually, in the industries and corporations, their stakeholders are educated and trained with required skills and techniques of management and organisation through special programmes. Though research is a form of creative knowledge, it is astonishing truth in India that, the HEIs which are very producers of knowledge do not provide any education and training to their stakeholders in the area of research. Therefore, the research education and training should be made mandatory for all the stakeholders in HEIs to increase the research literacy.

Introduction of Researchology

Another aspect of research education and training in the HEIs is 'introduction of Researchology' as a compulsory subject at all the levels of and for all the disciplines. In fact, research itself is a separate discipline of study in the faculty of Social Sciences. It has its philosophy, methods, theories and principles of study. It is a science as well as an art of theories, principles and practice based on certain assumptions alike other socio-economic sciences. It is not science of methods and techniques; rather it is an art of spirit, inspirations, enlightenment and intellectual actions. Methods and techniques are a part of research process, but, so far as higher education institutions are concerned, research has personality effect as it depends on the role, personality, qualities and competencies of the researchers.

Researchology is a new term in context of research, however, the terms 'research' and 'logy' are not new for the researchers and academics. The former denotes area of study and later denotes the science or system of study. In fact, both "area of study and system of study" are essential to denote the real meaning of any discipline. Generally, the term 'Research Methodology' is used in place of Researchology. However, in Research Methodology the area of study is methods and not 'research' as the term methodology denotes science of methods and not of research. Here, research becomes secondary and methodology becomes dominant. So far as Researchology in higher education is concerned, it is a system of study of research. It is to be noted that, in higher education, research is important and not

the methods and techniques. Therefore, 'research' should be the area of study as a separate discipline in the course-curriculum of HEIs. The renowned authors of the book 'Research in Education,' Best, J.W. et al. (2018), warns as, "Remember that research is essentially and intellectual and creative activity. The mastery of techniques does not confer research competence, though these skills may the creative problem solver to reach his or her objectives more efficiently." Conventional methodological perspective focuses on employing methods and techniques which is a mechanical and monotonous approach in the process of research. In its true sense, research is process of critical thinking, analysis of observations, organising thoughts, in-depth study, correlating the facts, drawing inferences and interpretations, establishing relationship and generalisation. In fact, all these cognitive and intellectual skills of the researcher affect the results of the research to a great extent.

Conventionally, the scientific procedure of research is termed as Research Methodology. But, Methodology is not research, as it denotes the procedure of employing the methods and techniques. However, Research in higher education is not only the procedure of employing methods and techniques. Identification and definition of problem, setting the objectives, formulating the hypothesis, research design are not subject to any methods and techniques in the process of Research. Research is not a science of methods, but it is a line of progressive steps in order to complete the process undertaken. The misconception of research with methodology has caused to generate indifference about education and applications of research in the mind of stakeholders in the HEIs and mainly among the faculty and students. In fact, research is a living phenomenon and methodology is a mechanical procedure. Methodology denotes two terms: (i) Methods and (ii) system of study. Thus, Methodology becomes the science of methods. Here Methodology becomes significant as a separate system of study and the main part 'research' becomes a subordinate or an auxiliary endeavour. Further, the research is not a science of methods; research itself should be understood as the science or system of study. Obviously, the term Methodology seems to be inconsistent in the theory and practice of research. Therefore, the science of Research can best be expressed by the term *"Researchology."*Researchology may be defined as a system of study of research procedure and applications. It has its own systematic, academic and orderly procedure of evolving knowledge, theories, laws and principles. Literally, the word 'Researchology' has two parts: *(i) Research* and (ii) *Logy*. The term *Research* means systematic study of a subject or problem to seek the truth or new knowledge or to investigate problems. The term *Logy* means science or system of study. Researchology in higher education is a discipline of multi-disciplinary nature in the faculty of Social Sciences. In India, in particular, the research or Researchology is not prescribed for study as a separate discipline, instead the methodology is studied to conduct research projects and write research papers. That is why, the faculty and students of research concentrate on methods and techniques and research part is overlooked. Therefore, Researchology should be introduced as a mandatory discipline of study in the course-curriculum of all faculties at UG and PG level programmes.

Branches of Researchology

Researchology is not one and the same for all the areas of study. There are various branches of Researchology in higher education classified according the subject or area of Research. The branches of Researchology include Researchology of Physical Sciences; Researchology of Chemical Sciences (Organic, In-organic and analytical); Researchology of Biological Sciences (Botany, Zoology, Health and Medical Sciences0; Researchology of Mathematical Sciences (Mathematics, Statistics); Researchology of Agricultural Sciences (including Dairy Science, Seri-culture, Horticulture, Flory-culture, Fishery Science); Researchology of Environmental Sciences (Ecology, Air, Water, Forest, Soil, Natural Resources, Sustainability of life); Researchology of Engineering Sciences (Civil, Electrical, Energy, Mechanical, Electronics, Computer, Aeronautical: Researchology of Geological or Earth Sciences; Researchology of Marine Sciences, Oceanography and Oceanology); Researchology of Social Sciences; Researchology of Administration; Researchology of Office Services; Researchology of Management and so on. Thus, according to the branch, the Researchology should be included as a compulsory discipline of study in each and every course-curriculum at UG and PG levels in all the faculties and streams of courses.

Research System in HEIs: Research System means application of research in the system of the HEIs and hence, is a form of institutional research. It is broader process of research extension from individual to institution. In fact, here

institution means the HEIs and the HEIs means their stakeholders. Therefore, research system in the HEIs is concerned with the stakeholders including top leaders, administrative personnel, academics, faculty and students. All these stakeholders have to adopt research as a system of organisation and operations of the HEIs. This research system consists of a cyclic process of thinking, observations, knowledge, experience, enlightenment, analysis, interpretations/inferences, search of alternatives, conclusions, decisions and actions. It is a systematic and orderly procedure of investigation of problems for innovative solutions. The research helps the stakeholders to think like a rebel and research starts with rebelling against present situation of pessimistic approach, indifferent attitude, static mind-set and stagnation of thinking. It provokes a new way of thinking which leads to change and vibrancy in the present situation. Thinking out of box, raising questions, visualizing problems and providing solutions are the implications adoption of research by the stakeholders in the HEIs. The research system works in the HEIs as the management system works in the industries and corporations. It is dedicated to practice of research practice at all levels and in every role of the stakeholders in the HEIs. It means "recognising research as an essential qualification for all the individual stakeholders and making its 'Teaching, Training and Practice' compulsory for human resources in all the disciplines and departments by structuring a suitable course according to their roles, duties and capacities". It is a process of developing mandatory practice of research by inculcating research habits through critical thinking, analytical mind-set, awareness and observations, detection of problems, providing alternative solutions according to role and capacity of the human resources in the Institutions of higher education.

Levels of Research System

Research System for Top Leadership: The research system helps the top leadership to judge the performance, to evaluate the functioning, to control the activities and to correct the deviations by alternative course of actions. This system has three essential dimensions as Policies, Planning and Strategies. The role of top leadership and management is making dynamic policies, planning the course of actions and formulating strategies of implementation. Getting things done is the main function of top leadership and management in the Institutions. Research helps in maintaining updated data bank, seeking regular information, categorising and analysing the information, interpretation, inferences and conclusions for further policies, planning, strategies and actions. System of Research also helps in policy evaluation, performance planning, building strategies, accounting of resources, provision of resources, efficiency audit of all resources, optimum utilisation of all resources. It also encourages involvement and participation of human resources, work-culture, educational environment, management by shared visions, team leadership, proactive and synergetic approach, role based autonomy with accountability, delegation of responsibility and authority, management of health, hygiene and cleanliness, green and pollution free premises, maintenance of discipline, peace and cooperation within the HEIs. There are two main wings of an educational institution viz.- (i) Administrative wing and (ii) Academics. The top leadership implement their policies, planning and strategies through these two wings. The research system helps them to control, direct, motivate and activate the activities of Administrative and Academic wings to achieve the desired objectives and goals of the Institutions.

Research System for Administrative wing: It is now recognised that, research is significant for students, faculty, academic leaders and policy makers. However, primarily, it seems extraneous and irrelevant to make research mandatory for all the persons including sweepers, cleaners, peons, clerks, sports trainers and administrative staff. Here, we have to understand the true nature and various levels of research. Research is not always a formal process or a set of certain methods and techniques. Research, basically is a mind-set, attitude, approach and intellectual endeavour. Every person in the HEIs, in any role and capacity, can learn to be dynamic always, face the adversities, strive continuously, select the correct course of actions and achieve the predefined objectives and goals. Continuous practice of research properly and suitably becomes the habit of life which ultimately builds the Research Character in every person in the HEIs. The research system helps the Administrative wing to think of: (i) providing efficient office services; (ii) institutional sensitivity, accountability, accessibility; (iii) self-discipline, duties and conduct; (iv) records of performance and non-performance; (v) creating cooperative work-culture and (vi) maintaining educational environment.

Research System for Faculty: In modern educational system, research is the most relevant and significant aspect for the Faculty of all disciplines and subjects. The research system helps the Faculty: (a) to study the subject prescribed for their teaching; (b) to understand all the aspects of the subject thoroughly, collect related information, facts about the contents and concepts and get in depth knowledge of the subject; (c) to prepare a teaching plan as per the time schedule allotted to him; (c) to plan for teaching aids, ICT equipment; (d) to adopt suitable pedagogy of participative teaching, students' involvement, group discussions, question-answer, tutorials, seminars, workshops etc.; (e) to teach the course curriculum as per planning with timely improvements; (f) to play a role of a mentor, facilitator and guide of the students; (g) to evaluate the performance of the students by adopting multi-stage evaluation methods and (h) to comply with the administrative work as an employee of the Institution.

Research System for Students: The students are the centre of all activities in the HEIs hence; they are main target of the research system. The research system for students involves the following activities:
(i) Research Teaching as a compulsory subject for all disciplines includes**:** (a) theoretical aspects - relevance and importance of research in life; (b) procedural aspects - how to think, how to study, how to observe, how to make notes, how to analyse, how to interpret, how to draw inferences and conclusions etc. and (c) research as a method of study - how to study the prescribed curriculum; how to prepare notes from references and text books; how to analyse the problems and questions; how to construct the solutions and answers.
(ii) Research Training includes: (a) step by step research process with trans-disciplinary approach - identification and definition of problems; inculcating research habits - critical thinking, analytical mind, close observations, seeing alternative aspects; (b) communication skills; creative visualisation, innovative ideas and (c) primary practical aspects of research - writing research articles - format and construction small research projects on surrounding problems - research questions, research objectives, hypothesis, gathering facts and arranging facts, analysis of data, drawing conclusions, writing of reports - format and step by step drafting of research articles.
(iii) Research Practice includes: (a) exposure and practical training - research internship - seminars, workshops, preparation of PPTs, research presentation, research publications; (b) research evaluation, self-improvements, writing research papers, presentation and publication; (c) projects on local/regional/ national problems creating societal impact; (d) trans-disciplinary approach in selecting problems, collecting data and drawing conclusions; (e) dissertation submission with due significance in grading the results; (f) creative writing and communication skills – authorship of texts; (g) industrial interactions, understanding environment, visits, reports, projects, internships; (h) commercial and trade visits– study of market, exchange, consumer behavior and (i) research projects as a part of curricula, undertaking funded or non-funded research projects.

Systemic Research in the HEIs

Systemic Research means, the research in the living system in an institution of higher education as 'life blood in the body of its organization'. In simple words, Systemic Research means "making research as a part of the system of the Institution". Research is not in Articles, Papers or Projects. It is in the mind, thinking, observations, analysis, approach, attitude, behaviour and habits of the persons working in the Institution. It is in the decisions, actions and inactions of the persons involved in day to day functioning of the Institution. Therefore, Systemic Research means the process of developing of all the Human Resources in an Institution by inculcating research habits and building research character of every one of them. For everybody in the Institution, research helps in realising capacity and responsibilities of their roles and duties and also the ways of doing them as best as possible with utmost abilities and best level of efforts. For every human being, research helps in personal SWOT analysis of Institutional role and responsibilities, setting of daily, weekly, quarterly, yearly targets and objectives with the long-term goals and making plans accordingly for execution with determination, dedication and consistency. The success means a step forward towards achieving the predefined targets, objectives and goals with consistent improvement in efforts, qualities and efficiency.

In the present system most of universities and colleges, the Faculty, Staff and Administrative heads are most of the time busy with counting the number of years of their service and time of further up-gradation of scales and posts

and also with calculating the increased salaries and allowances. They also make a show of success on the basis of whatever they receive on the criteria of period and post. This is a system, as presently it is, of getting authority, seniority and salaries in relation to the period and posts. In the present system, whatever results are achieved, those are only because, they have to maintain and sustain this system with a fear of losing the seniority, posts and salaries. This system creates the mentality of "we are doing our job." This is the situation of static mentality of personnel and stagnation of institutional life which restricts or prevents further improvements, progress and expansion. This is the peculiar example of present institutional system where the Systemic Research becomes relevant and significant.

The Systemic Research can be understood through practical examples of Institutional life. A receptionist in any institution is not merely a doll or idol to do Namaste! She or He is the first Public Relations Officer to receive and welcome the stakeholders on behalf of the Institution. She or He is the first eye of the Institution to keep watch on the happenings in front of the door and around. This is an approach to recognise the relevance and significance of the role, capacity and responsibilities of the receptionist. In the same way every person in the Institution has his or her own course of duties, responsibilities and a role as an indivisible organ of the Institution. Even the students also have their roles and responsibilities and their own course of actions related learning, study and seeking knowledge. In the same way, from the watchmen at the gate of the Institution to the peons, clerks, supervisors, Faculty, departmental heads, administrator/registrar, Principal/Dean/Director and ultimately the persons at the top of the organisation have their roles, duties, and responsibilities and the course of actions to be followed accordingly.

The process of Systemic Research creates "win-win" situation for every person in the Institution. For example, the role, capacity, responsibility and relevance of a receptionist must be realised by the receptionist herself or himself on one hand and the staff, Faculty, administrators and leadership should recognise these factors in the role of the receptionist on the other hand. This process happens reciprocally with every role and for every person mutually and collectively. In Systemic Research everybody feels and experience that his or her role is properly recognised and respected; his or her efforts are suitably recognised and achievements or contributions are properly honoured and timely rewarded. Thus, Systemic Research results in motivation of human resources with the sense of participation and achievement in their role and capacity in the institution. It also creates sense of affiliation and fulfilment among the human resources and enlightens them with the purpose of life, helps to build the character of conviction to the duty, institutional integrity, and professional ethics.

Research Outcome in the HEIs

The holistic research system is mainly meant for the stakeholders in the HEIs including top leadership, persons in management and administration, staff, academics, faculty and students. Therefore, the outcome of this holistic research system is expected among these stakeholders only. This system will enhance productivity and efficiencies among these stakeholders which will lead to better results at all the levels in the HEIs. This system will help to bring change and transformation in the HEIs through the practice of values including conviction and commitment; professional ethics; world class quality of infrastructure and education; participative management; shared vision; team work; cooperative work-culture; educational environment; amenities of health and hygiene and green premises. The systemic will help the top leadership to activate the academic and administrative wing to achieve the qualities of progressive and consistent policy making, effective strategies, human resources development, provision of infrastructure and equipment, shared leadership approach, common cause, collective mission, delegation of responsibility and authority with strict discipline and professional ethics, suitable rewards and proper honour and recognition of special and extraordinary contribution or achievement, significance of merit, quality and transparency in all the spheres of the institution, due care of sustainability, green campus, environment of teaching, learning, development and discipline, social sensitivity and responsibilities.

This system will motivate the Faculty to inculcate research habits and nurture character of self-discipline, punctuality, proactive teaching approach, role of a mentor and facilitator, regularity in classes, well-preparation of delivery of knowledge, effective communication skills, self-improvement in pedagogy, advance teaching plan, short-

term and long-term plan of career enhancement, quest for knowledge, creation of knowledge, specialisation, comprehensive study approach, contribution to subject knowledge, teaching with new ideas and innovations, goal setting, time-management, determination, consistency in efforts till satisfactory results. In addition to successful completion of prescribed course-curriculum, the systemic research ignites and encourages the students to inculcate the qualities of self-discipline, punctuality, regularity in attendance, self-realization of career development, quest for knowledge, creative visualisation, innovative and progressive approach, communication skills, social relations, research and study, specialisation, comprehensive study approach, learning with understanding approach, personal SWOT analysis, ambition, goal setting, time management, academic performance, determination, consistency in efforts till satisfactory results.

Research Monitoring in HEIs

Research monitoring refers to a formal system of controlling and monitoring research operations through statutory framework and central authority. Monitoring also includes funding provisions, mentoring through timely guidance and coordination of activities through central authority. So far as higher education in India is concern, the monitoring, control and guidance aspects of the HEIs are entrusted to the University Grants Commission (UGC) and Ministry of HRD, Government of India. The State Universities, Colleges and Institutes in higher education have additional monitoring and control from Higher Education and Technical Directorate of the States. However, the theory of holistic research system is suggested as a sustainable research system at the level of HEIs, and therefore, the monitoring and mentoring is expected at the levels of HEIs through a Central Board or Committee of experts appointed for that purpose. Though the theory of holistic research system assumes to start with existing resources and budget, this system needs regular monitoring and mentoring at the level of HEIs. A regular system of monitoring, funding provisions and guidance is essential to achieve the desired goals of HEIs. Therefore, it should be established on top priority as a sustainable mechanism to provide comprehensive and inclusive solution to the problems of HEIs.

Research Assessment in HEIs

Research assessment refers to periodical review, performance evaluation, note of deviations and corrective actions through alternative solutions in the functioning of the holistic research system at the levels of HEIs. In fact, there is no sustainable system of evaluation for assessment of research performance in higher education institutions in India. As stated by Pinar, M. et al. (2020), “Research assessment exercises have been increasingly used to assess the quality of research produced by higher education institutes (HEIs). These assessment exercises are mainly used to allocate research funds selectively to high performing HEIs, increase accountability for the use of public funds, track the research progress of the HEIs and create performance incentives for HEIs and researchers.” This research article of Pinar, M. et al. (2020) reveals that United Kingdom has established a system of yearly assessment of research performance of HEIs through Research Excellence Framework (REF) exercise. However, in India, there is no such system of evaluation established for assessment of research performance of the HEIs at the level of University Grants Commission and Ministry of HRD, Government of India.

There is National Institutional Ranking Framework (NIRF) in India run under Ministry of HRD, Government of India. However, this framework outlines a methodology to rank institutions across the country on the basis of overall recommendations and broad understanding arrived at by the Core Committee to identify the broad parameters for ranking various universities and institutions. The parameters broadly cover Teaching, Learning and Resources, Research and Professional Practices, Graduation Outcomes, Outreach and Inclusivity, and Perception. However, this ranking framework does not include assessment of research system at the levels of HEIs. This model of holistic research system assumes a regular mechanism of research assessment of research performance at the levels of HEIs. The research assessment in HEIs includes the assessment of research performance regarding academic research, research education, research system, systemic research and practical application of research by top leaders, administrative staff, faculty and students. This process of research assessment also covers the evaluation of utility, implications, viability and sustainability holistic research system in the HEIs.

CONCLUSION

The HEIs in India are facing various problems of lack of research support, ignorance of national character, lack of integrity to common cause, indifference towards accountability, status-quo mind-set of stake-holders, and stagnancy of prospects, lack coordinated efforts, and under-utilised resources. The problems in the HEIs in India are chronic in nature and are evolved because of historical background and socio-cultural ecosystem which need a comprehensive and inclusive solution. The model of holistic research system is a generic way to provide solution to these problems within the available resources and budget. The problems in the institutions of higher education are not of funding or physical resources, but, pertain to cognitive and intellectual aspects and therefore, their solution lies in human spirit and inspirations to strengthen systemic research in the HEIs and enlighten national character among stake-holders through coordinated efforts and collective leadership for common cause; institutional research system, inculcating research habits and nurturing research character in the institutions.

Many plans and schemes have been implemented by Governments through Ministry of HRD and UGC, new HEIs established, funds provided, salaries increased and many attempts have been made for development of HEIs, however, except increase in number of institutions with new enrolment of students, the qualitative improvements could not take place and the predetermined goals of HEIs could not be achieved. The holistic research system is the proper and suitable way to end the situation of stagnancy and saturation in the HEIs in India through realisation of role and responsibilities among stakeholders at institutional level and enlighten them with the spirit of purpose of life, common cause and national character. Research is an applied science and hence, only teaching-learning of research and granting of some projects is not enough to achieve the desired goals. The real goal of research system is application or utilisation of research knowledge and skills in the respective roles and responsibilities in the HEIs. The holistic research system will work as a parallel to the system of management in industries and corporations through educational entrepreneurship and application and utilisation of research will nurture proper approach and attitude and inculcate research habits and research character among stake-holders at all levels in the HEIs.

REFERENCES

1. Angom, Sangeeta (2015), Private Universities in India- Growth Status and Concerns (Occasional Paper), National University of Educational Planning and Administration, New Delhi, India, p. 52.
2. Best, J.W.; Kahn,J.V.; Jha,A.K. (2018), Research in Education, Pearson India Education Services Pvt. Ltd. Noida (UP) India, Pp.22 & 25.
3. National Education Policy (2020), Ministry of HRD, Government of India, Website: www.mhrd.gov.in.
4. Pinar, Mehmet and Unlu, Emre (2020), "Determinant of Qualify of Research Environment: An assessment of the environment submissions in the UK's Research Excellence Framework in 2014" Research Evaluation 2020, 1-14, doi:10.1093/reseval/rvaa003, Oxford University Press.
5. Report (2013), 12th Five Year Plan (Vol.2, 2012-17), Planning Commission of India. Website: www.niti.planningcommission.gov.in; Pp. 89-123.
6. Report (2018), All India Survey of Higher Education (AISHE 2018-19), Ministry of HRD, Government of India, New Delhi. (website: www.aishe.gov.in)
7. Sharma Kavita A.(2013), Sixty Years of the University Grants Commission - Establishment, Growth and Evolution, University Grants Commission, New Delhi, India, p. 340.
8. STRIDE (2019), Scheme for Trans-disciplinary Research for India's Developing Economy Operational Guidelines, University Grants Commission, New Delhi, Website: www.stride.bhu.ac.in.

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RESEARCH ARTICLE

Applications of Group Theory in Molecular Systems Biology

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ABSTRACT

Group theory has applications in material science, science, and software engineering, and even riddles like Rubik's Cube can be solved utilizing group theory. The group theory is playing a significant role in the current day of science, arithmetic and statistics. It was determined in the nineteenth century in relationship with conveying answers for arithmetical articulations. Specifically, the group was the arrangement of the relative multitude of changes of the underlying foundations of a mathematical articulation that shows the attributes that the blend of any two of these stages has a place with the set. Also, later on, the conviction was made summed up to the thought of an abstract grouping. Notwithstanding, an abstract group is the study of a set, with an activity characterized on it. In this paper we discuss some selected mathematical points that can assist us with bettering comprehend the limit among living and non-living frameworks. In the topic molecular systems biology we discuss the abstract algebra and group theory. All through the present work we quickly portray conceivable issues. Regarding the hereditary code we recommend that it could be conceivable to utilize perturbation hypothesis to investigate the neighboring potential outcomes in 64-D space time complex of genome which is advancing. Concerning logarithmic chart hypothesis, there are a few minor open issues we examine. Comparable to arrange elements and groupoid formalism we recommend that the organization chart probably won't be the principle center in gaining the knowledge on aggregate yet the stage-space of the organization elements. In this paper we explain a basic instance on network of C6 and its stage-space organization. Let's imagine that sub-atomic organization ofs cell is really an unpredictable organization of hyper cycles and input circuits that could be better spoken to in a higher-dimensional space. We guess that focusing on hubs in the atomic organization that have key parts in the stage space, as uncovered by investigation of the automorphism deterioration, may be a superior method to medicate revelation and therapy of disease.

Keywords: group theory, molecular systems biology, groupoid formalism, C6 network, automorphism deterioration

INTRODUCTION

In the year 1944 there was a publication of Erwin Schrödinger on what life is. This little book was a significant motivation for an era of scholars in physics in entering the field of natural chemistry and microbiology, with the objective of endeavoring to characterize existence by methods for material science and science. In spite of the fact that a huge measure of the work is being concluded. Lets consider an instance, the particular manner by which science course books catalog the vital qualities of the life- to depict it from non-living issue- incorporates digestion, self-support, duplication including hereditary material and advancement by regular determination. This spellbinding methodology doesn't address the true intricacy of life forms, the dynamical character of environmental frameworks, or the topic of how the aggregate rises up out of the genotype. (Example: for infection measures). This cosmos could be seen as an enormous Riemannian resonator in which advancement happens through cycles of energy dispersion and decrease in entropy. The survival could be considered as a portion of hardware the cosmos is using to decrease in inclinations of energy. The development comprises of bit by bit balance measure which is breaking, in which the energy thickness distinction comparative with the encompassing is lessened. At the point the cosmos was framed 13.7 billion years back through the Big Bang, a sequence of unconstrained events of breaking occurred, which advanced into a heterogenous structure from the uniform quantum vacuum we are able to notice this day. Truth be told quantum vacillations of early cosmos got exploded into cosmological scales, through a cycle called enormous expansion, and remainders of the quantum changes could be noticed straightforwardly in a variety of the inestimable microwave foundation radiation in various ways. In each of the stage along development of cosmos- from quantum gravity, to essential bits, molecules, primary cluster of stars, worlds, the group of planets, there is a further the uniform quantum vacuum balance the uniform quantum vacuum. The cosmological, heavenly, and nuclear molecule reflections can be capably communicated in expressions of group theory.

It additionally booted that establishment of all among present day material science depends upon group theory. Four (4) principal communications (powers) in nature: solid (answerable for dependability of cores in spite of the shock of emphatically protons which are charged), feeble (showed in beta-rot), gravitational and electromagnetic. Initial three were depicted by quantum hypotheses: a SU (3) check bunch for quarks, and a SU(2)×U(1) hypothesis for bound together electro-feeble communications. Through the hypotheses we could infer, considering the instance, Electromagnetism from maxwell's hypothesis, which is premise of electrical designing which is contemporary and the photonics, including activity of laser. The group theory gives the structure to developing models or analogies from deliberations, and for the control of those reflections to plan new frameworks, make new forecasts and put forward new speculations. Motive of the present work is looking at arrangement of elective in numerical deliberations empirical to science, and specifically systems biology. Balance and breaking balance assume the conspicuous job in formative science, to radially symmetric creatures from bilaterians. Cohen, Woese and Streams all have called for more profound examinations of survival by applying new numerical reflections to science. Point of the present work isn't such a great amount to discourse the difficult inquiry embossed by Schrödinger, yet to grow a arrangement of numerical methods conceivably appropriate to coordinating the enormous measures of information accessible in the post-genomic period, and in a roundabout way add to tending to the difficult inquiry. In this paper our main focus is on inquiries of atomic systems biology utilizing numerical methods in space of theoretical polynomial math which to this point have been to a great extent disregarded by specialists.

Group Theory

Group theory is a part of unique variable based math created to examine and control conceptual ideas including symmetry. Prior to characterizing group theory in explicit expressions, it would assist with beginning with an illustration of a theoretical idea, a rotation group.

Consider a card which is a square in 3-D area, we could pivot it π radians, that is, 180 degrees, in coordinates of X, Y and Z; let's speak to the turns by (r1, r2, r3). Lets's likewise consider do nothing activity shown by the letter e. On the

off chance that we pivot the card with r1 and next with a r2 rotation, at that point we come by what could be compared to doing just a r3 turn. We would thus be able to round out a Cayley table (likewise called "augmentation" table, however the activity isn't standard increase). The balance regarding askew in Cayley's table reveals that the group is an abelian group: if pivots are acted two by two, they will be commutative, i.e.. rm rn = rn rm.

As a form of matrix the group operations can be written:

$$E = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad R_1 = \begin{bmatrix} 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad R_2 = \begin{bmatrix} -1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -1 \end{bmatrix} \quad R_3 = \begin{bmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

Presently the situation is to express this conventional meaning of group G: group is nonempty set containing binary function (indicated by * here) that fulfills accompanying conditions:

Associative Law:

$$\text{for all } a,b,c \in G,\ (a * b) * c = a * (b * c).$$

Identity Law:

$$\text{There is an identity element } e \in G, \text{ such that } a * e = e * a = a \text{ for all } a \in G.$$

Inverse Law:

$$\text{For any } a \in G \text{ there is an element } b \in G \text{ such that } a * b = b * a = e.$$

Contingent upon the quantity of components in set G, let's discuss regarding groups which are finite as well as groups which are infinite. Arrangement of finite simple groups has been done; the order perhaps been the best accomplishment mathematical science of twentieth century. In science finite groups likewise have inescapable applications, going to sub-atomic orbitals from gem structures, and as itemized beneath, in the systems biology. Sn and Zn are most remarkable among the finite groups, n is +ve number. Sn, symmetric group which is the set, is assortment of changes of bunch of n components, whose number of components, i.e., order, n!. Incidentally, any group which is finite is a subgroup of a group which is finite for a value of n. Sn has cyclic group, Zn as its subgroup of comprising of permutations which are cyclic. There are two different introductions of Zn:

[1] By multiples of $2\pi/n$, the rotations.

[2] The numbers module n of group.

Infinite groups are more enthusiastic to examine, yet that are having extra structure, like some structure of complex or of topological space, where the extra structure with the group structure is viable, is likewise arranged. Quite compelling are the Lie groups, which are at the same time topological spaces and groups, and the inverse operation and multiplication in groups are functions which are continuous. Totally characterized groups are lie groups, a significant number among them are emerging as matrix groups. The portrayal of matrix permits to utilize matrix algebra which is conventional based math to control the objects in the group, however doesn't assume any unique job. Truth be told any group, infinite or finite, to any subgroup of matrix groups, is isomorphic. This is the domain of group representation theory.

O(n), orthogonal groups (here n is an integer) are produced using n by n matrices which are real orthogonal, i.e., the n × n matrices O for which

$$O^{-1} = O^T$$

$$OO^T = I.$$

The orthogonal group which is special of SO(n) comprises of the matrices which are orthogonal whose determinant is +1, and it structures a subgroup of orthogonal group: SO(n) $\subset$ O(n). Mathematically, the particular orthogonal group SO(n) in n dimensional Euclidian space is group of rotations, the orthogonal group O(n) furthermore holds the reflections also

Likewise, $U(n)$, unitary matrices,

$$U^H = U^{-1}$$

$$U^H U = I$$

Structure the group (H is complex conjugation of every component of matrix along with the transposition). SU(n), special unitary matrices, fulfill the condition det(U) = +1 limitation, and groups are formed. At last, we notice that "symplectic" or Sp (2n) groups, however specified the way that they are difficult to characterize, it won't show a proper explanation here. It would be indicated, that lattice groups are utilized in portraying the "buildup" of heriditary code. Other significant definition that we can experience includes groupoids. A groupoid is broader that group, which also comprises of couple (G,μ), here G is a set of components, for instance, arrangement of integers Z, and μ is binary operation again typically alluded is "augmentation," yet not be mistaken for math multiplication in any case, binary operation μ isn't characterized to each pair in the set G. Groupoids are helpful in portraying organizations, and accordingly interactome and transcriptome networks.

Heriditary Code

Here, lets survey few works portraying hereditary code in groupoid and group theory expressions. One can without much of a stretch envision hereditary codes dependent on protein or RNA, or mixes thereof. At the point the hereditary code "consolidated" from the "cosmos of conceivable outcomes" there are numerous potential balance which is breaking occasions.

The codon can be spoken to as a component in the immediate result of 3 indistinguishable sets, S1= S2= S3= {U, C, A, G}:

$$S1*S2*S3 = \{U, C, A, G\} * \{U, C, A, G\} * \{U, C, A, G\} = \{UUU, CCC, AAA, ..., GGG\}$$

The cross product which is triple has 4*4*4 = 64 triplets possible. We know, the table which is three-way product contains repetition of the code. This was completely during the 1960s worked out, in the absence of group theory, utilizing experimental information on the atomic structure of bases.

The direct way in depicting hereditary code includes symmetry of code doublets. Neubert and Danckwerts utilized this klein group; which is abelian group containing 4 components, which is isomorphic to the symmetry of non-square shape in 2-space. Our goal is to depict symmetry of code doublets utilizing Klein group. We can segment the arrangement into two subsets of dinucleotides:

$$M_1 = \{AC, CC, CU, CG, UC, GC, GU, GG\}$$

$$M_2 = \{CA, AA, AU, AG, GA, UA, UU, UG\}$$

The M1 doublets could coordinate with third base for trio that shows no impact on coded amino corrosive. The M1doublets are related with ruffian trios. Doublets in M2 don't code for amino acids with no information in the trio on the third base. Presenting the operators of doublet exchange (e,α,β,γ) we could play out the accompanying the base exchanges:

$$\alpha : A \leftrightarrow C \quad U \leftrightarrow G$$

$$\beta : A \leftrightarrow U \quad C \leftrightarrow G$$

$$\gamma : A \leftrightarrow G \quad U \leftrightarrow C$$

The exchange logic is given below: α trades purine bases with non-reciprocal pyrimidine bases, β trades correlative bases which could go through some changes in hydrogen bond, and γ trades purine with another purine and pyrimidines with another pyrimidines, and is a structure of α with β. Identity operator is e.

Jungck and Bertman stretched out the klein portrayal to product of Cartesian group (K4×K4), brought about four D hypercube, called tesseract. The sides of solid shape are sets of operators from klein group and hereditary code for doublets.

The sides of the hypercube of dinucleotides form two octets, two sets M2 and M1. These vertices of every Octet lie on planes of consistently associated area. Such area M1 has appeared in shaded region of the above diagram. These Octets are neither cosets nor subgroups of the subgroup. Both of them are unaltered under the operations (β,e) and (e, e). The 2 Octets could likewise be inter-changed by following up on one of them with (γ,α) and additionally (α,α).

All in all, very little could be expressed regarding result of the 2 groups. On the off chance that K has the subgroups A and B, at that point item could possibly be the subgroup of K. Regardless, result of the 2 sets might be significant and prompts the idea of the cosets. Leave the group K alone the klein group K={e,α,β,γ} and has the subgroup H={e,β}, at that point the left coset is αH={αe,αβ}={α,γ}. As K is an abelian group, privilege coset Hα={eα,βα}={α,γ} and can discover that αH=Hα. Coming up next are 4 cosets of (K4×K4) operators of hereditary exchange:

$$H_1 = [(e,e) : AA, (\beta,\beta) : UU, (e,\beta) : AU, (\beta,e) : UA]$$
$$H_2 = [(\beta,\gamma) : UG, (e,\alpha) : \overline{AC}, (\beta,\alpha) : \overline{UC}, (e,\gamma) : AG]$$
$$H_3 = [(\beta,\gamma) : \overline{GU}, (\alpha,e) : CA, (\gamma,e) : GA, (\alpha,\beta) : \overline{CU}]$$
$$H_4 = [(\gamma,\alpha) : \overline{GC}, (\alpha,\gamma) : \overline{CG}, (\gamma,\gamma) : \overline{GG}, (\alpha,\alpha) : \overline{CC}]$$

We composed relating dinucleotide close to operator in format (e,e):AA, and so forth; the bar over the dinucleotides shows enrollment in an alternate octet of totally codons which are degenerated, whereas other dinucleotides are the vague codons.

(K4×K4), hypercube portrayal which is 4-D in the figure above proposes that 64 components in hereditary code, trios, can be spoken to by hypercube which is 64 D and evenness activities in the space can be codons. Normally it is possible to shape triple item to show up at 64 D hypercube as overall hereditary code. Obviously various vertices of the hypercube code for a similar amino corrosive. That is supposed to be Surjective guide, since there is more than one nucleotide trio codes for a similar amino corrosive. In the year 1982 Findley portrayed further evenness break down of group D, and take different subgroups which are isomorphic including the klein group and depict elective coding plans in the hyperspace. In previous paragraphs we depicted the hereditary code as for intrinsic symmetries. In the year1985 Findley proposed that 64-D hyperspace, D, might consider as a data space; in the event that one incorporates time (advancement), at that point we also have a 65-D data space time complex. This current hereditary code developed on differentiable complex, M[X]. Developmental directions in space are proposed to be the geodesics in data space time. It ought to be conceivable to utilize measurable strategies to register the distances between the species (polynucleotide directions) by utilizing a measurement, state Euclidean measurement:

$$d = \left[\sum_{\mu} (x'^{\mu} - x^{\mu})^2\right]^{1/2}$$

To reproduce directions in this space from phylogenetic tree. It ought to be conceivable to subsequently see areas of the data space time that haven't been investigated on advancement. One can theorize on the code-direction by getting hypothesis of Stuart Kauffman on nearby conceivable by the perturbation hypothesis. The bends on the complex have to plan, in an unpredictable way, to balance breaking bifurcation or depicted below and hence give second course to the Findley's differential math. The other way in dealing with understanding development of hereditary code depends on analogies with molecule material science and the symmetry parting from higher dimensional space. Forger Hornos and use bunch hypothesis to depict the advancement of hereditary code from the higher dimensional space. Actually, they proposed dynamical framework polynomial math or Lie polynomial math, Lie polynomial math is structure conveyed by digression space at personality component of Lie group. Beginning with sp(6) algebra of Lie, the accompanying chain of the breaking of symmetry would bring about current hereditary code with redundancies:

$$sp(6) \supset sp(4) \oplus su(2) \supset su(2) \oplus su(2) \oplus su(2) \supset$$
$$su(2) \oplus u(1) \oplus su(2) \supset su(2) \oplus u(1) \supset u(1)$$

The underlying symmetry of sp(6) breaks into 6 subspaces sp(4) and su(2). The sp(4) at that point parts to su(2) and su(2) while second su(2) factors to u(1).

Multi-Nucleated Cells and Cell Cycle

Cell cycle is illustration of characteristic use of the group theory in light of cyclic symmetric governing cycle. Procedure in cell cycle incorporate G1→S→G2→M, later again to G1. Sometimes G0 is basically so short as to be nonexistent so we will overlook that state. To project cell cycle into the terms of group theory recollect meaning of group we have given before. Only sensible methodology for projecting cell cycle into group theory is to utilize symmetries of the square. To the cyclic group Z4 it is isomorphic and Abelian. We get by composing rotation activities for cell cycle as stages:

$$R_0 = \begin{pmatrix} G1 & S & G2 & M \\ G1 & S & G2 & M \end{pmatrix}$$

$$R_{90} = \begin{pmatrix} G1 & S & G2 & M \\ S & G2 & M & G1 \end{pmatrix}$$

$$R_{180} = \begin{pmatrix} G1 & S & G2 & M \\ G2 & M & G1 & S \end{pmatrix}$$

$$R_{270} = \begin{pmatrix} G1 & S & G2 & M \\ M & G1 & S & G2 \end{pmatrix}$$

For instance R 90 could be communicated as mapping:

$$G1 \rightarrow S$$
$$S \rightarrow G2$$
$$G2 \rightarrow M$$
$$M \rightarrow G1$$

This table of the cell cycle group recommends investigating group operations of the genuine control of the cells. Johnson and Rao directed examinations on moving cores from one cell into another to create cells with numerous cores. A fascinating inquiry they tended to is what impacts will the G2 core would have when relocated to cell whose core is in S stage? These tests were intended to address bigger inquiries regarding chromosome buildup and the guideline of DNA combination.

Microphotographs of binucleated HeLa cells.
B: A heterophasic S/G2 binucleated HeLa cell at t = 6 hours after combination and hatching with 3H-thymidine.
A: A heterophasic S/G2 binucleated HeLa cell at t = 0 hours after combination.

A portion of cores are pre-named with the name 3H-thymidine to improve perceivability. Subtleties of investigations and outcomes could be found in the original works. Now lets inspect, by methods for group table, merged state or the binucleated cells. Normally it requires investment for "responses" to occur and for cell to settle to some steady attractor. At times more than one core is added to a cell in other state. For instance two G1 cores were added to a cell in the S stage. Johnson and Rao recorded speed to union. Consider an instance, if G2 core was added to cell in G1, there is basically no change. They are simply harsh perceptions; given sufficient opportunity, all cells would meet to the state M, the most grounded attractor in elements of the cell cycle. To show that this follows genuine definitions of group we have to show associativity and discover a character and backwards component, or, on the other hand, to show an isomorphism with a group which is known.

Abelian group is shown in the above table, commutativity consistently holds: $a \circ b = b \circ a$ for every one of the $a,b \in G$, where G is a group. We can likewise show associativity, $a \circ (b \circ c) = (a \circ b) \circ c$; for instance:

$$G1 \circ (S \circ G2) = (G1 \circ S) \circ G2$$
$$\Leftrightarrow G1 \circ S = S \circ G1$$
$$\Leftrightarrow S = S$$

$$G1 \circ (M \circ G2) = (G1 \circ M) \circ G2$$
$$\Leftrightarrow G1 \circ M = M \circ G2$$
$$\Leftrightarrow M = M$$

Then again, it is obvious from augmentation table we can't have the group structure on this given set {G1,G2,S,M}. To be specific, in the group G any column or row of augmentation table will contain components of G definitely once, henceforth will be a permutation of components. This property comes up short for the rows of M and S. Moreover, result of G1 and G2 is indistinct. In any case, the set {G1,G2,S,M} conveys structure of a groupoid. Comparative contemplations apply on the off chance that we combine cells of various sort, or separation state. These sorts of analyses were completed for various foundational microorganisms. Another combination type explore includes atomic exchange starting with one sort of substantial cell then onto the next, and deciding the character of result. The variation of this is to move RNA populaces among the cells and notice adjustment in cell's aggregate.

CONCLUSION

In this audit we have addressed a couple of numerical thoughts that might grow the comprehension of limit among non-living and living frameworks. In the part of the hereditary code we suggested that it might be conceivable to utilize perturbation hypothesis to investigate the adjoining prospects in 65-D space time complex of developing genome. One can begin by utilizing mappings of phylogen as verifiable information on the complex and register separates in the space. These insights of the distances might then be taken care back by means of perturbation hypothesis to contemplate direction. Obviously, we perceive that current best in class bioinformatics makes this proposition generally impossible as of now. Yet, unrefined diagrams of the procedure could be created.

REFERENCES

1. Annila A: Space, time and machines. 2009, arXiv:0910.2629v1 [physics.gen-ph]
2. Brooks R: The relationship between matter and life. Nature. 2001, 409: 409-411. 10.1038/35053196.
3. Cohen JE: Mathematics Is Biology's Next Microscope, Only Better; Biology Is Mathematics' Next Physics, Only Better. PLoS Biol. 2004, 2: e439-10.1371/journal.pbio.0020439.
4. Danckwerts HJ, Neubert D: Symmetries of genetic code-doublets. J Mol Evol. 1975, 5: 327-332. 10.1007/BF01732219.
5. Goh K-I, Cusick ME, Valle D, Childs B, Vidal M, Barabási A-L: The human disease network. Proc Natl Acad Sci USA. 2007, 104: 8685-8690. 10.1073/pnas.0701361104.
6. Penrose R: The Road to Reality: A Complete Guide to the Laws of the Universe. 2004, London: Jonathan Cape
7. Rosen R: Life Itself: A Comprehensive Inquiry into the Nature, Origin, and Fabrication of Life. 1991, New York: Columbia University Press
8. Schrödinger E: What Is Life?: The Physical Aspect of the Living Cell ; with, Mind and Matter ; & Autobiographical Sketches. 1992, Cambridge: Cambridge University Press
9. Weinberg S: The Quantum Theory of Fields. 1995, Cambridge: Cambridge University Press, 1:
10. Woese CR: A New Biology for a New Century. Microbiol Mol Biol Rev. 2004, 68: 173-186. 10.1128/MMBR.68.2.173-186.2004

Table 01: Group Theory

	e	r_1	r_2	r_3
e	e	r_1	r_2	r_3
r_1	r_1	e	r_3	r_2
r_2	r_2	r_3	e	r_1
r_3	r_3	r_2	r_1	e

Table 02: Heriditary Code

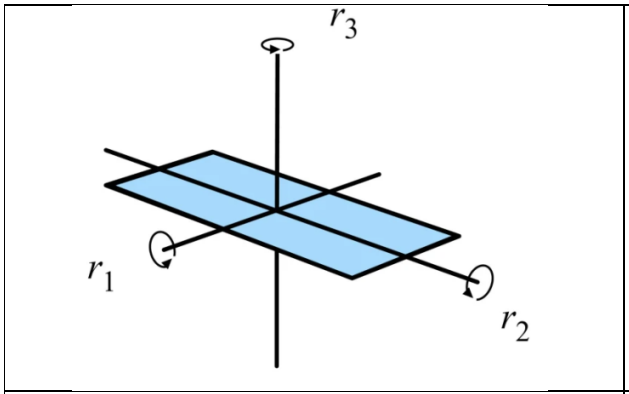
	e	α	β	γ
e	e	α	β	γ
α	α	e	γ	β
β	β	γ	e	α
γ	γ	β	α	e

Table 03: Multi-Nucleated Cells and Cell Cycle

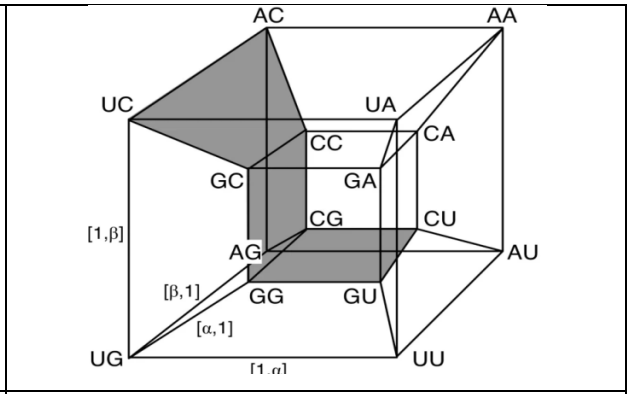
	G1	S	G2	M
G1	G1	S	G2	M
S	S	G2	M	G1
G2	G2	M	G1	S
M	M	G1	S	G2

Table 04: Multi-Nucleated Cells and Cell Cycle

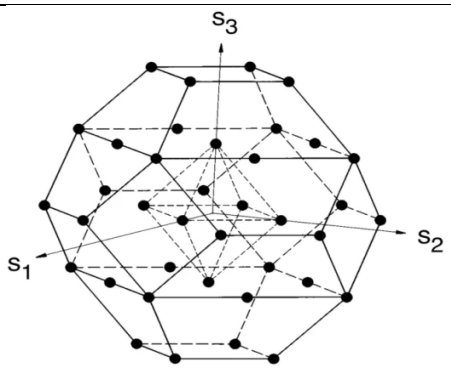
	G1	S	G2	M
G1	G1	S	G1/G2	M
S	S	S	S	M
G2	G1/G2	S	G2	M
M	M	M	M	M

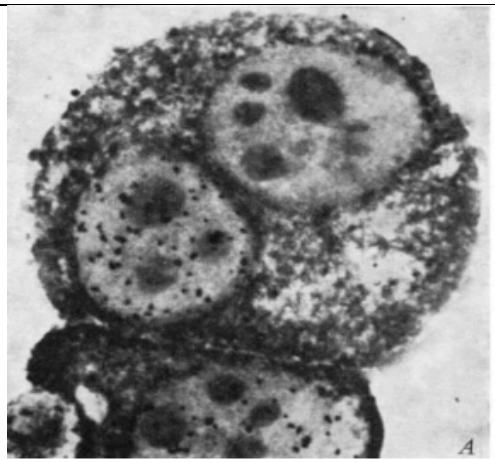


Group Theory

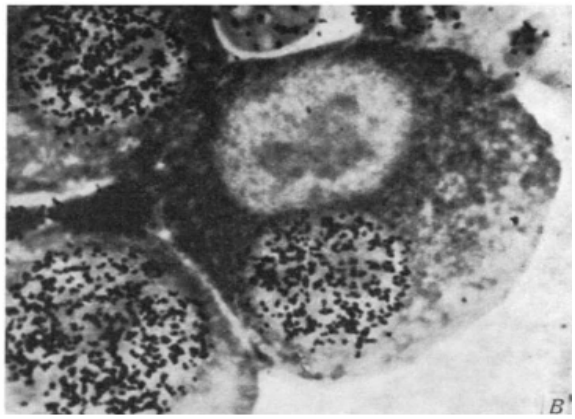


Doublet genetic code from ($K4 \times K4$) product.





A heterophasic S/G2 binucleated HeLa cell at t = 0 hours after combination.



A heterophasic S/G2 binucleated HeLa cell at t = 6 hours after combination and hatching with 3H-thymidine.

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RESEARCH ARTICLE

In silico Screening of Phytochemicals from *Nigella sativa* for Its Potential Therapeutic Roles Targeting SARS-Co-V-2

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ABSTRACT

In December2019, pneumonia caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Co-V-2) infection was discovered in Wuhan, Hubei Province, China. The infection is reported to have sprouted in China, but it quickly spread over the world: and was declared as the first coronavirus pandemic by WHO. The SARS-Co-V-2caused a new variant of coronavirus disease (COVID-19) outbreak is seeing a significant increase in affected individuals worldwide. Nonetheless, more than 1000 clinical trials focusing on the use and effectiveness of antiviral medicines as a prospective therapeutic treatment are now underway. The present study focuses on identifying the phytochemicals from the plant *Nigella sativa* (Black cumin) that is able to inhibit the activity of the enzyme Papain like protease, that is reported to be responsible for viral proliferation in SARS-Co-V-2. The molecular analysis was carried out using Autodock tool and PyMOL.

Keywords: pneumonia, antiviral, *Nigella sativa,* enzyme, coronavirus.

INTRODUCTION

A viral infection, now popularly known as Corona Virus Disease 2019 (COVID-19) was first identified in Wuhan, China, in the middle of December, 2019 which significantly spread throughout the world causing severe illness[1]. It was subsequently reported from Thailand, Japan followed by the entire globe. A huge population has got affected due to this disaster, taking lives of millions [2]. The Transmission rate of this infection has surged due to carelessness in adhering to the COVID protocol by the public. Also, globally all countries are mutually dependent for many requisites and hence are channelized for Import and Export purpose. This requires a mandatory communication between different communities which ultimately has led to an invisible bridge for spreading of the virus within the planet. As a transmissible disease the infection has spread almost into every age group and causes respiratory illness and many more symptoms.

Tyrell and Bynoe, who cultivated these viruses from patients with ordinary colds, were the first to characterise them in 1966[3]. Based on the shape, which is spherical virions with a core shell and solar corona-like surface projections, the virus was named as Coronavirus. Generally, the virus is enveloped and the Lipid envelope encases the helical capsid of Coronavirus. They have large, petal-like spikes radiating outwards from their envelopes, giving them a corona-like appearance. It is a positive strand RNA virus which contains plus strand genomes, genomes are polyadenylated and capped, allowing them to be translated once released into the cytoplasm. After releasing into the cytoplasm, the virus starts infecting the host organism and membrane fusion is mediated by protein during in the early stages of infection[4]. As the virus mostly habitats the respiratory tract, it causes complications of the respiratory tract but in some cases, it directs towards the gastro intestinal tract and other parts of the body as well. It was also found that 2–10% of COVID-19 patients experienced gastrointestinal symptoms such vomiting, diarrhoea, and abdominal pain. In 10% of patients, diarrhoea and nausea came before the onset of fever and respiratory symptoms [5, 6, 7].

There are no particular therapeutic strategies to treat this viral infection and to control the transmission. This mandates vaccination of each individual to prevent the world wide spread of the virus along with personal hygiene. SARS-Co-V-2 is reported to spread through the oral cavity, nasal cavity, and other mucous membranes when susceptible individuals come in contact with virus-containing bodily fluids (sputum, saliva, faeces) from humans or animals. Biological aerosols are droplets of pathogens (viruses or bacteria) suspended in the air for an extended length of time resulting in the possibility of disease transmission over great distances[8]. Hence there are so many ways of transmission of such infection and it is very hard to prevent the infection in an ideal way. Improving self-immunity to boost the physiological process of the individual to oppose the entry of virus in to the body may be a novel idea to stop the infection in a large extent.

From the prehistoric time it has been found that medicinal plants have been used as a source of medicine in practically all societies since time immemorial. According to epidemiological and animal research, eating fruits, vegetables, and whole grains on a regular basis lowers the incidence of chronic diseases [9]. These scavengers are strategically segregated in the cells to provide optimal intracellular protection and offer first line to third line defence activity. Any non-toxic food extract supplement with scientifically proven health benefits for the prevention and treatment of various diseases is referred to as a nutraceutical [10]. The secondary metabolites that is present in plant extract is known as phytochemical, which plays a key role to protect the organism from invading pathogens. Polyphenols, flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, and fibres are among the Phytochemicals / phytonutrients described as potentially having health advantages[10]. Pharmacologically these secondary metabolites are nutritive and enriched with antioxidants to protect the organisms from diseases. Phytochemical research based on ethnopharmacological data is widely regarded as a successful method for discovering new antioxidant and anti-infective compounds in higher plants[11].

Based on the immense health benefits, herbal medicines can be used as an alternative to treat symptoms caused by COVID-19, as many traditional herbs have demonstrated antiviral characteristics. A large number of herbs have the capability to reduce the risk of getting infection, such as *Nigella sativa* (Black cumin). *N. sativa* is explored in traditional medicines from millennia to cure a variety of ailments, including asthma, common cold, headaches, nasal congestion, rheumatic diseases, warts, and more. It is also known to have effect on infections, cancer, diabetes, hypertension, obesity, cardiovascular illnesses, and gastrointestinal problems [12]. The current study aims to project a specific phytochemical from *Nigella sativa* (Black cumin) that can prevent the symptoms of COVID-19, which is caused by the SARS-Co-V-2 virus, by inhibiting a protein pathway that can govern viral proliferation.

MATERIALS AND METHODS

Software used

In this paper, we provide a PyMOL plugin that enables molecular docking, virtual screening, and binding site analysis. The plugin acts as a bridge between PyMOL and Autodock Vina, a popular docking application. With visual support from PyMOL and a graphical user interface, the plugin allows to complete the entire docking studyto successfully analyze the molecular interaction process.

List of phytochemicals

The prefix “phyto-” in phytochemicals comes from the Greek word phyto, meaning “plant.” Phytochemicals are plant chemicals and are non-nutritive compounds. Human and other organisms are constantly exposed to a range of oxidising chemicals, some of which are required for life to exist. These agents can be found in air, food, and water, or they can be created by cellular metabolic activity. To maintain optimal physiological conditions, it is critical to maintain a balance between oxidants and antioxidants. Overproduction of oxidants can contribute to oxidative stress, particularly in persistent bacterial, viral, and parasitic diseases. To overcome such conditions antioxidants should be consumed which are mostly found in the phytochemicals. According to published studies, the herb *Nigella sativa* (Black cumin) includes a variety of phytochemicals, including cravacol, 4-terpineol, limonene etc [13].

Enzymes found in SARS-Co-V-2

Energy is essential for survival of an organism, which is generated by metabolic activities within the organism. The regulation of certain metabolic processes necessitates the use of enzymes to complete the reaction. As a result, enzymes play a critical role in an organism's life cycle. The RCSB database was used to find the enzymes that control the lifecycle of the virus. SARS-Co-V-2 Papain like protease, with the protein data base number 6W9C has been reported to be responsible for the virus's proliferation mechanism, which is crucial for the virus's survival.

Molecular Docking

Phytochemicals isolated from plants, work as ligand molecule by forming a strong covalent bond with the viral protein to successfully inhibit the microorganism. Autodock vina software was used to perform the molecular interaction process to analyse the binding affinity in between the ligand molecule and receptor. For analysing the interaction process, the structure data file of the phytochemical of *Nigella sativa* (Black cumin) was downloaded from PubChem website which acts as a ligand in the interaction process. The enzymes data base was collected from RCSB website which acts as receptor molecule in the interaction. The size and position of this binding site is depicted in PyMOL and can be tweaked interactively. Docking can be launched straight from PyMOL after defining the binding site and receptor- ligand preparation. Interaction maps are used by Autodockduring docking. The analysis of these maps is calculated by auto grid, prior to the actual docking run. After completion of the docking process the affinity value is generated through which binding strength is calculated. The higher the negative value the stronger the binding strength.

RESULTS AND DISCUSSION

The autodock tool is an automated docking program, that is used to dock compounds. Through the plugin, docking process generated by docking applications can be easily loaded into PyMOL. Meta data comprising the docking score is provided in a tiny text viewer for each docking, allowing easy investigation of configuration/scoring connections. The most stable docking model for each molecule was chosen based on the Autodock scoring function's best-scored conformation prediction. Fig 1: Depicts the enzyme's active site, which provides a better attachment for the ligand molecule, resulting in more precise binding strength.

The interaction of 4-terpineol- Papain like protease has the largest positive value of-CDOCKER energy, as shown in Table 1. According to the analysis from the tabulation it is clearly found that the 4-terpineol phytochemical of *Nigella*

sativa has the ability to successfully interact with the viral enzyme and inhibit the proliferation process which ultimately help in degradation of the virus.

CONCLUSION

Nigella sativa (Black cumin) has been found to have anti-covid-19 effects to a certain extent. The purpose of this study was to figure out what phytochemicals was responsible for the therapeutic effects. A molecular analysis was done by using the PyMOL and Autodockvina tools to recognise the phytochemical 4-terpineol which has potential to interact with the enzyme Papain like protease. And it was concluded that 4-terpineol has a strong binding interaction or affinity with the enzyme Papain like protease. Thus, the presence of phytochemicals 4-terpineol in the plant *Nigella sativa* provides therapeutic capabilities against SARS-Co-V-2 virus.

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REFERENCES

1. Paules CL, Marston HD, Fauci AS. Coronavirus infections-more than just the common Cold. JAMA 2020, 323: 707-708
2. WHO, World Health Organization. https://www.who.int/. Accessed April 14, 2020
3. Tyrrell DAJ, Byone ML. Cultivation of viruses from a high proportion of patients with colds. Lancet 1966, 76-77.
4. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ. Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses. 2012, 770-783.
5. Paules CL, Marston HD, Fauci AS. Coronavirus infections-more than just the common Cold. JAMA 2020, 323: 703-704.
6. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. JAMA 2020, 323: 1061-1069.
7. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020, 395: 507-513.
8. Shi Y, Wang G, Cai XP, Deng JW, Zheng L, Zhu HH, et al. An overview of COVID-19. JZUSAM 2020, 21: 343-360.
9. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary Polyphenols and the Prevention of Diseases. Critical Rev. Food Sci. Nutr2005,45: 287-306.
10. Prakash D, Gupta C, Sharma G. Importance of Phytochemicals in Nutraceuticals. J. Chin. Med. Res 2012, 1: 70-78.
11. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern. Med 2006, 635.
12. Mollazadeh H, Afshari AR, Hosseinzadeh H. Review on the Potential Therapeutic Roles of *Nigella sativa* in the Treatment of Patients with Cancer: Involvement of Apoptosis. J Pharmacopuncture 2017, 20: 158-172.
13. Tembhurne SV, Feroz S, Sakarkar DM. A review on therapeutic potential of *Nigella sativa* (kalonji) seeds. J Med Plants Res 2014, 8: 166-167.

Table 1: Result of molecular interaction

Name of the ligand	Binding sequence	Affinity (kcal/mol)
4-terpineol	1	-6.7
	2	-6.7
	3	-6.3
	4	-6.2
	5	-6.2
	6	-6.1
	7	-5.8
	8	-5.8
	9	-5.8

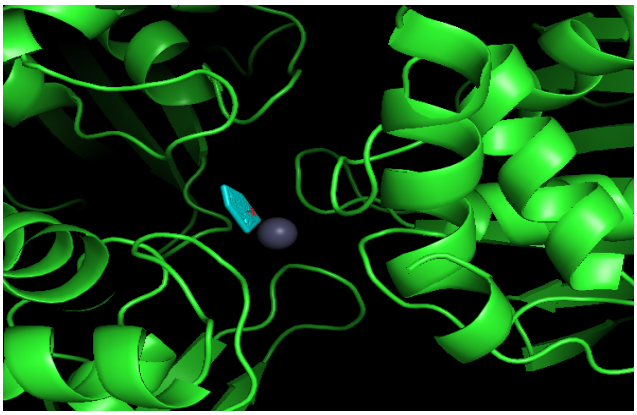

Fig 1: Active site of enzyme Papain like protease

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RESEARCH ARTICLE

IoT Based Smart Irrigation System using Node MCU

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ABSTRACT

Internet of Things (IoT) transforms the agricultural economy also enables farmers to face enormous challenges. In fact, Agriculture is the backbone of our economy. IoT-based farming has numerous advantages over traditional farming, designed to enhance agricultural output and environmental effects. These techniques require ongoing monitoring and automation to operate properly. IoT will aid in increasing productivity and reducing water consumption while requiring less human work. Using IoT helps to forecast agricultural production in various areas, including soil temperature, humidity, moisture, pH value, motion detection water levels, and the optimum timing of plant to be delivered to plug, which might assist in boosting crop yield. This paper focuses primarily on the framework of the system that focuses on the sensing of soil quality, moisture, the prediction of the field irrigation requirements using several environmental parameters, as well as the weather forecasts that support the crop growth and provide sufficient humidity with limited human interaction. Decisions are made via to microcontroller. When a variation from the expected values exists via the Blynk App notice, the user is recognized in the field. In addition to the soil parameters of this project. This ensures the health of the crop.

Keywords : Node MCU, Automatic irrigation System, Arduino Sensors, Blynk app

INTRODUCTION

Internet of Things able to connects the physical devices interconnected with computer devices, digital and mechanical devices, humans or animals, things that, without any human input, can sensibly collect and transmit data over the Internet. Everything has a unique identification. It is an advanced examination and automated framework that employ detection, organization, massive information, and human-made awareness innovation to provide whole management frameworks. IoT really extend the Internet beyond smartphones and PCs. IoT have change the modern world . Smart homes, smart cities, smart irrigationsystems, each and everything has to be turned into a smart with the help of IoT. It also has applied in various field like agriculture etc.

Following are the Special Features of IoT,

1. Less power: These plays a major role in designing an electronic system are high performance and common battery usage.
2. Cloud computing:Cloud computing is a method of storing data remotely over the Internet. Maintenance, management, backup, and availability over a network.
3. Network connection: Internet is a must for IoT devices, Internet connectivity is required for communication, and each physical object is allocated an IP address.

Circuit Configuration

The design of Smart irrigation systems involves several components, including Node MCU , Soil Moisture Sensor, PH Sensor, Temperature Sensor, Moisture Sensor, Relay Module, Blynk Cloud, and Submersible Pump. It allows the user to automate the process of irrigation based on humidity levels and weather like rain. The system comprises a pump that pumps water based on environmental conditions, such as soil and rain humidity. The pump is connected to the Node MCU, yet the Node MCU cannot provide an output voltage above 3.3V from its GPIO. We need a 5V relay module. The three sensors are connected to the Node MCU. The Internet connection makes Node MCU to Blynk app which the date is transmitted and collected in the form of graph.

Working

Five sensors are used in this arrangement to collect data from the field/farm. This data is delivered to Node MCU, which subsequently executes the commands specified in the code. A12V regulated power supply powers the Node MCU. It performs many roles depending on the land's environmental circumstances, Different crops necessitate varying soil moisture and temperature and humidity conditions. So, when the soil loses humidity to less than 50%, the motor will automatically turn on to pump the water, and it will continue to do so until the moisture in the water reaches 55%, at which point the pump will be turned off. The sensor data will be relayed to the Blynk Server at predetermined intervals, allowing it to be watched from anywhere in the world. All of the data is then combined in a graphical format in the Blynk App for further study.

Component Used

The following materials are used in this project

Node MCU

One of the key features of the Node MCU board is the highly integrated chip. It can host the programme or offload all Wi-Fi networking operations to another application processor. Due to Node MCU's robust on-board processing and storage capabilities, it can be easily integrated with sensors and other application-specific devices via its GPIOs. As a result of its excellent on-chip integration, the system, including the front-end module, takes very little PCB space.

The Node MCU development board a true plug-and-play solution for inexpensive projects using WiFi. The module arrives pre-flashed with Node MCU firmware so they're ready to go just install your USB driver (below). Node MCU WIFI Dev Board Internet Of Things board contains a full WiFi module with all the GPIO broken out, a full USB-serial interface, and a power supply all on the one breadboard-friendly package. This board is pre-flashed with *Node MCU* – a Lua-based firmware for the Node MCU which allows easy control via a neat scripting language – Lua .so you're ready to go in just a few minutes. The ESP-12 Lua Node MCU WIFI Dev Board Internet Of Things is an all-in-one microcontroller + WiFi platform that is very easy to use to create projects with *WiFi* and IoT (Internet of Things) applications. The board is based on the highly popular WiFi Module chip. This WiFi development board already embeds in its board all the necessary components to program and upload code. It has a built-in USB to serial chip upload codes, 3.3V regulator, and logic level converter circuit so you can immediately upload codes and connect your circuits.

Soil Moisture Sensor

Soil Moisture Sensor is a simple findout for measuring the moisture in the soil and similar materials. The soil moisture sensor is pretty straightforward to use. The two large exposed pads function as probes for the sensor, together acting as a variable resistor. The more water in the soil means, the better the conductivity between the pads will be and will result in lower resistance and a higher SIG out. Soil Moisture Sensor functioning all you will need is to connect the VCC and GND pins to Node MCU, and we receive a SIG out, which will depend on the amount of water in the soil.

Rain Drop Sensor

It is used for raindrops detection. It is also for measuring rainfall density. The rain sensor can be used for all kinds of weather monitoring and translated into output signals. The rain sensor can monitor a variety of weather conditions and turn into several fixed output signals and Analog outputs. As raindrops are collected on the circuit board, they create paths of resistance that are measured via the op-amp. the lower the resistance, the lower the voltage output. Conversely, the less water, the greater the output voltage on the analog output.

Temperature and Humidity

It is an electronic device that measures the temperature of its environment and converts the input data into electronic data to record monitor. There are many different types of temperature sensors. it can get a variety of Temperature & Humidity, including a Digital Microcomputer Thermostat Switch, a Humidity Controller Module, a high-temperature resistance Probe, Moisture Sensor, and many more modules. Temperature Sensor applications in many industries. In many applications where maintaining a specific temperature is vital there the temperature sensor is used. For example, if products must be kept at a certain temperature like agriculture, the responsiveness and accuracy of the temperature sensor are critical.

PIR Motion Sensor

The Passive Infrared Sensor (PIR) sensor module is used for motion detection. It is often referred to used "PIR", "Pyroelectric", "Passive Infrared" and "IR Motion" sensor. The module has an on-board pyroelectric sensor, conditioning circuitry and a dome shaped Fresnel lens. It is used to sense movement of people, animals, or other objects. They are commonly used in burglar alarm. The sensors are able to detect a change in temperature from object to ambient from as 3°C. Changes in the level of detected emission are evaluated by a window comparator, and if the change exceeds the upper or lower threshold the output level of the sensor goes high and can be used to trigger external circuitry.

pH Sensor

This is Liquid pH Value Detection Sensor for Node MCU. The pH stands for the power of hydrogen, which is a measurement of the hydrogen ion concentration in the body. This is used in Water quality testing and Aquaculture. The total pH scale ranges from 1 to 14, with 7 considered to be neutral. A pH less than 7 is said to be acidic and

solutions with a pH greater than 7 are basic or alkaline. The PH electrode has a single cylinder that allows direct connection to the input terminal of a pH meter, controller, or any pH device. The pH electrode probe is accurate and reliable that can give almost instantaneous readings.

Software Used

Arduino IDE

Various kinds of Arduino boards are available depending on system needs. However, all Arduino boards have one thing is common, they are programed through the Arduino IDE. Inputs and outputs of an electronic system such as speed, voltage, and size can differ widely. Some boards are designed to be embedded and have no programming interface, which you would need to buy separately. Some can run directly from a 3.7V battery, others need at least 5V. Arduino is an open source platform based on hardware and software. ArduinoIDE - a ready-made software used to upload the computer code to the board and program the circuit board - consists of a physical board and a board that can be programmed.

The key features of Arduino IDE are,

i) Arduino boards are able to read analog or digital input signals from different sensors and turn it into an output which, connect to the cloud and many other actions.
ii) Your board can be controlled by sending instructions via the Internet to its microcontroller through Arduino IDE.
iii) Unlike most previous programmable circuit boards, Arduino does not need an extra piece of hardware in order to load a new code onto the board via USB cable.
iv)Additionally, the Arduino IDE uses a simplified version of C++, making it easier to learn to program.
v) Finally, Arduino provides a standard form factor that breaks the functions of the micro-controller into a more accessible package.

Blynk APP

Blynk was designed for the Internet of Things. It can control hardware remotely, it can display sensor data, it can store data, vizualize it and do many other cool things.

There are three major components in the platform:

- **Blynk App** –It's allows you to create and interfaces to the projects using various widgets. The main goals of Blynk App is interface between human and machine.
- **Blynk Server**–It is responsible for all the communications between the smartphone and hardware. You can use Blynk Cloud or Blynk server locally. It is open-source software, could easily handle thousands of devices and can even be launched on a Node MCU.
- **Blynk Libraries** - Enables server communication and executes all incoming and outgoing commands from your Blynk app and hardware.

Every time you press a Button the Blynk, where it magically finds its way to your hardware. It works the same in the opposite direction and everything happens like a blynk of an eye.

RESULT

The graphical depiction of the gathered real-time data from the field can be seen. As seen in the figure, we can view the temperature and humidity measurement in real-time and its figure below. The pH level of the soil sample is indicated on the LCD screen, and soil moisture is low because the soil moisture is low. The submersible pump begins pumping the water into the field until the appropriate humidity is reached.

CONCLUSION

The given model analyses the usage of IoT in agriculture. This model seeks to increase crop output by helping to anticipate optimal crop sequence for a given soil. Blynk app help to collect the soil in real-time, and hence the gathered data can be used further to analyze the crop. At different times of the day, we also measured soil moisture, temperature, and humidity, as well as environmental pH. Cloud data storage also helps farmers improve production, evaluate waste, and infections in the field. This system is economically efficient and viable. It also aims to optimize water resources to tackle problems such as water scarcity and maintain sustainability. IoT in agriculture is the focus of this model. This paper will propose solutions to improve agricultural methods, stimulate production and increase the efficiency of using limited resources.

Future Aim

Artificial Intelligence to the current technology can further make this project more innovative and forecast climate conditions and human actions. This intelligent system will also aid in understanding the defects and repair them since suggestions would be provided in the system. The current system works with the Wi-Fi system but may be modified further using the GSM module. For communication between the hardware and the cloud, the GSM module requires a sim card. If an entire town uses the method, the topographical circumstances would be easy to understand. Utilizing these techniques, the whole village would be easier to comprehend the soil quality in the area and plan the crop. This project would be significant in agricultural growth.

REFERENCES

1. Rao, R. N., & Sridhar, B. (2018). IoT based smart crop-field monitoring and automation irrigation system. *2018 2nd International Conference on Inventive Systems and Control (ICISC)*. doi:10.1109/icisc.2018.8399118.
2. Monica, M., Yeshika, B., Abhishek, G., Sanjay, H., &Dasiga, S. (2017). IoT based control and automation of smart irrigation system: An automated irrigation system using sensors, GSM, Bluetooth and cloud technology. *2017 International Conference on Recent Innovations in Signal Processing and Embedded Systems (RISE)*. doi:10.1109/rise.2017.8378224.
3. IoT Based Smart Irrigation Monitoring & Controlling System in Agriculture. (2020). *International Journal of Recent Technology and Engineering Regular Issue,8*(6), 2436-2440. doi:10.35940/ijrte.e6851.038620.
4. Kumar, A., Kumar, A., Singh, A. K., &Choudhary, A. K. (2021). IoT Based Energy Efficient Agriculture Field Monitoring and Smart Irrigation System using Node MCU. *Journal of Mobile Multimedia*. doi:10.13052/jmm1550-4646.171318.
5. Dheer, A., Sharma, M. L., &Tripathi, K. (2020). Smart irrigation system based on IoT. doi:10.36227/techrxiv.13361093.
6. Purnawan, P. W., & Rosita, Y. (2019). RancangBangun Smart Home System MenggunakanNode MCU Esp8266 BerbasisKomunikasiTelegram Messenger. *Techno.Com,18*(4), 348-360. doi:10.33633/tc.v18i4.2862.
7. Nugroho, A., Hendrawan, A., Pinem, A. P., Cholil, S. R., &Hidayanti, N. (2020). Designing automatic system smart curtains using Node MCU based on android. *Engineering, Information and Agricultural Technology in the Global Digital Revolution,* 112-114. doi:10.1201/9780429322235-23.
8. Chavda, R., Kadam, T., Hattangadi, K., &Vora, D. (2018). Smart Drip Irrigation System using Moisture Sensors. *2018 International Conference on Smart City and Emerging Technology (ICSCET)*. doi:10.1109/icscet.2018.8537377.
9. Panigrahi, S. (2020). SMART FARMING: IOT Based Smart Sensor Agriculture Stick For Live Temperature And Humidity Monitoring. *SSRN Electronic Journal*. doi:10.2139/ssrn.3651933.
10. Bentabet, D. (2020). Cloud-IoT Platform for Smart Irrigation Solution Based on Node MCU. *Artificial Intelligence and Renewables Towards an Energy Transition Lecture Notes in Networks and Systems,* 689-696. doi:10.1007/978-3-030-63846-7_65.

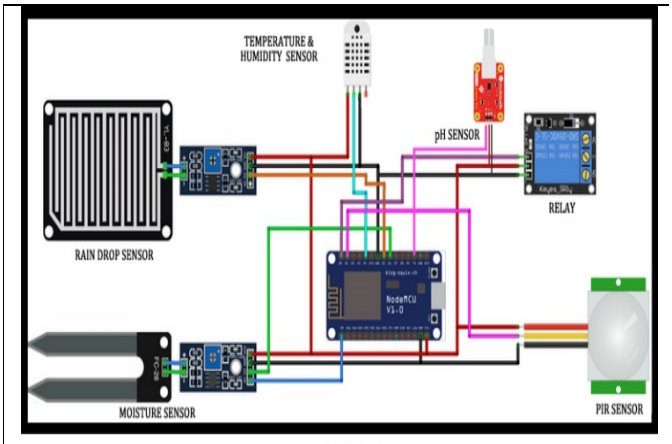


Fig.1.Circute Diagram

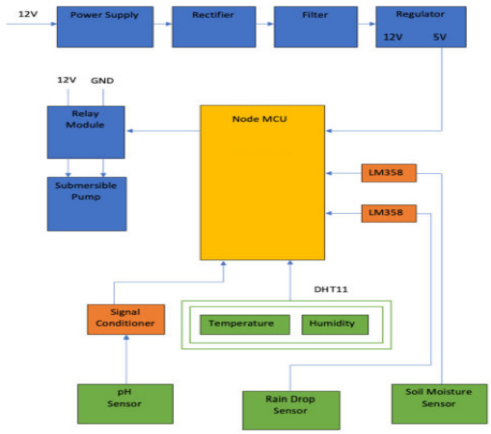
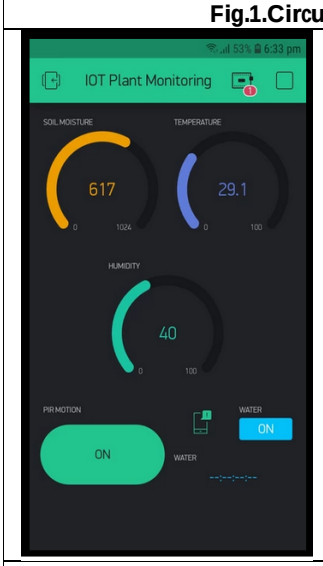


Fig.2. Node MCU



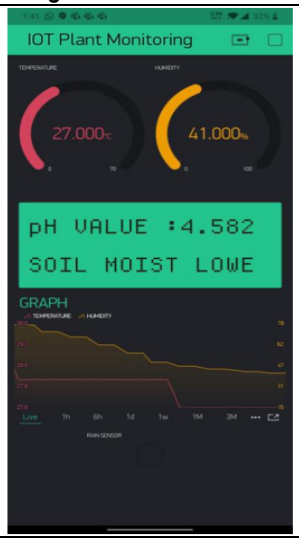


Fig.3.Graphical Representation

Fig.4. Rain Sensor Active

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RESEARCH ARTICLE

Wound Healing Activity of Phytochemical Coumarin against Alkaline Protease from Pseudomonas aeruginosa: An In silico Approach

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogenic bacterium considered to be an common cause of delayed wound healing and had caused significant morbidity and mortality. In burn patients, *P. aeruginosa* is a prevalent nosocomial infection, and multi-drug resistance strains are common in burn units, resulting in a high fatality rate. Bacterial proteases are a broad and diversified collection of proteases produced by all bacteria and have a wide range of physiological and biochemical functions. Through the turnover of unfolded proteins in the host environment and the proteolysis of regulatory proteins in response to environmental cues, Gram-negative bacteria's intracellular expression and external secretion of proteases are important contributors to infection. Coumarin has huge antimicrobial properties against microorganisms, infections, protozoa, parasites and yeasts. In this current investigation, alkaline protease (PDB ID 1akl) of *P.aeruginosa* was recovered from PDB site and utilized as target protein. Phytochemical coumarin structure was additionally recovered from Pubchem. Autodock vina programming was utilized to examine the communication between alkaline protease and coumarin. We reasoned that, phytochemical coumarin is powerful against alkaline protease chemical of *P. aeruginosa*. It tends to be utilized for drug discovery.

Keywords: Coumarin, Alkaline protease, Wounds, *Pseudomonas aeruginosa*, Autodock

INTRODUCTION

In primary closure, traumatic wounds, burns, and chronic skin ulcers, infection is the most common cause of delayed wound healing. *Pseudomonas aeruginosa* is an opportunistic pathogenic bacterium that causes significant morbidity and mortality while posing a management challenge (Nagoba et al. 2013). The release of inflammatory cytokines is linked to tissue damage at a concentration of 105 bacteria per gram of tissue (Haji Zaine et al., 2014). Gram-positive

(e.g., *S. aureus*) and Gram-negative (e.g., *P. aeruginosa*) bacteria react to the hostile environment by forming a biofilm that protects them from the immunological response of the host (Jenkins et al. 2014). Excessive antibiotic use can encourage the selection and proliferation of resistant isolates, which is part of the problem. In burn patients, *P. aeruginosa* is a prevalent nosocomial infection, and multi-drug resistance strains are common in burn units, resulting in a high fatality rate. *P. aeruginosa* colonises 14-33 percent of burn sites within 10 days of admission, making it the most common cause of bacteremia in burn patients (Xu & Hsia, 2018). The surface area of the burn (>30 percent total body area), previous disease, or difficulties prohibiting surgical excision of the eschar or wound closure all increase the risk of infection in burn patients (Turner et al., 2014). Antimicrobial activity must be balanced. The key to prevention and treatment of burn wound sepsis is rapid and thorough debridement. *P. aeruginosa* enters the subcutaneous lymphatic system, multiplies, and invades the surrounding healthy tissue (Gonzalez et al., 2016). Because systemic medicines are ineffective in weakly perfused avascular tissue, topical antiseptics are frequently utilised to aid in the eradication of *P. aeruginosa* wound and burn infections. The antibacterial activity must be harmonised with the tolerability.

Plants offer enormous potential in the management and healing of wounds. Many plants are utilised as traditional remedies to heal various wound injuries and skin problems (Lingaraju et al., 2012). *Cinnamomum zeylanicum* is a commonly used spice and flavouring agent, and is a natural insecticide, is an antimicrobial, antidiabetic, antilipidemic, anti-inflammatory, and neuroprotective agent (Rao and Gana, 2014). Coumarin (1,2-benzopyrone)(Figure 1), found in *C. zeylanicum* is a volatile phenolic substance consists of fused Benzene and α-pyrone rings and in normal temperature it resembles like a white crystal having a melting point of 341k-344k. Researchers found, coumarin compounds having antimicrobial effects, antimutagenic properties, anti-inflammatory properties. In addition, the simplicity of its chemical backbone is very attractive, as well as the reactivity of the benzene and pyrone rings. Conjugated double bonds are responsible for an electronic environment that plays a very important role in this family of compounds. Coumarin compound has the potential to fight against bacterial pathogens as well as the soil-borne fungalpathogens, plant fungal pathogens and human fungal pathogens such as *Candida albicans*, *Candida tropicalis, and Aspergillus fumigates* (Hu e al. 2017).

Bacterial proteases are a broad and diversified collection of proteases produced by all bacteria and have a wide range of physiological and biochemical functions. Through the turnover of unfolded proteins in the host environment and the proteolysis of regulatory proteins in response to environmental cues, Gram-negative bacteria's intracellular expression and external secretion of proteases are important contributors to infection. There is mounting evidence that bacterial proteases have a role in epidermal adhesion and penetration. The alkaline protease (AprA) of *P. aeruginosa* was discovered to cleave the C-terminal laminin G domain-like modules of laminin chains, the basement membrane's main glycoprotein. The cleavage of these laminin modules, on the other hand, produced physiologically active peptide fragments with antibacterial characteristics and the ability to induce wound closure by increasing keratinocyte migration and proliferation (Suleman, 2016).

Autodock Vina was used to perform molecular docking in this research. Molecular docking is a computational technique that aims to predict the supported path of a ligand to its macro molecular objective (receptor) while they are coupled to one another to form a stable state. This method was used to identify phytochemicals from the plant extract, which act as a ligand and form a firm covalent link with the bacterial protein, effectively suppressing the pathogen (Ismail and Uzairu, 2019). For recognising sub-atomic collaboration and performing sub-atomic docking, the Auto dock, an open-source protein-ligand docking programme, was used. The purpose of this work was to assess the action of coumarin by blocking the alkaline protease pathway (Figure 2) in *P. aeruginosa.*

MATERIALS AND METHODS

AutoDock Vina (Trott and Olson, 2010) was used for analysis. AutoDock Vina is an open-source program used for

the purpose of molecular docking. It was designed and employed by Dr. Oleg Trott in the Molecular Graphics Lab at the Scripps Research Institute. Molecular docking technique has been utilized to distinguish the phytochemical from the plant extract, coumarin, which used as a ligand and formed a solid covalent bond with the bacterial protein to effectively restrain the organism. Docking was performed in AutoDock Vina (http://vina.scripps.edu/), which predicts interactions between ligands and proteins. Results were seen and examined with PyMOL variant 2.3.2.

RESULTS AND DISCUSSION

From the Autodock molecular docking examines, a decent ΔG Kcal/mol esteem was obtained for the compound coumarin. The acquired docking results (Figure 3) affirmed the ability of the selected compounds in terms of effective molecular interaction with the examined protein, targeted compound coumarin was determined to have more grounded affinities in binding with the active site of alkaline protease. The docking consequences of the compounds with most elevated binding energy were obtained to be – 5.4 kcal/mol (Table-1). This lesser value of ΔG indicates the successful approach of phytochemical coumarin as a drug against emerging bacterial pathogen of *P.aeruginosa*. Because many infectious organisms have gained resistance to conventional medications such as antibiotics, they no longer respond to them, medicinal plants are becoming increasingly important. Herbal treatments are often more effective and safer than pharmaceuticals. Because they are non-toxic, they can be given for an extended period of time (Vinothapooshan and Sundar, 2010). Both common cinnamon and cassia cinnamon have been assumed to be generally harmless when consumed and can be an effective drugable agent against pathogenic Gram negative bacterial actions to prevent complicated wound infections (Farahpour et al. 2012).

CONCLUSION

From the above study it has been concluded that using Autodock vina software, molecular docking operation result indicated that the phytochemical, coumarin has an effective interaction with the vital enzyme, alkaline protease enzyme of the *P. aeruginosa.* It was found that coumarin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe.

REFERENCES

1. Nagoba BS, Selkar SP, Wadher BJ, Gandhi RC. Acetic acid treatment of pseudomonal wound infections: a review. J Infect Public Health. 2013; 6(6):410-5.
2. Haji Zaine N, Burns J, Vicaretti M, et al. Characteristics of diabetic foot ulcers in Western Sydney, Australia. J Foot Ankle Res. 2014; 7(1):39.
3. Jenkins TC, Knepper BC, Jason Moore S et al. Comparison of the microbiology and antibiotic treatment among diabetic and nondiabetic patients hospitalized for cellulitis or cutaneous abscess. J Hosp Med. 2014; 9(12):788-94.
4. Xu Z, Hsia HC. The Impact of Microbial Communities on Wound Healing: A Review. Ann. Plast. Surg. 2018; 81:113–123.
5. Turner KH, Everett J, Trivedi U, Rumbaugh, KP, Whiteley M. Requirements for Pseudomonas aeruginosa Acute Burn and Chronic Surgical Wound Infection. PLoS Genet. 2014; 10: e1004518.
6. Gonzalez MR, Fleuchot B, Lauciello L, Jafari P, Applegate LA, Raffoul W, Que YA, Perron K. Effect of Human Burn Wound Exudate on Pseudomonas aeruginosa Virulence. mSphere 2016; 1: e00111–e00115.
7. Lingaraju G, Krishna V, Joy Hoskeri H, Pradeepa K, Venkatesh Bab PS, et al. Wound healing promoting activity of stem bark extract of *Semecarpus anacardium* using rats. Nat Product Res. 2012; 26(24):2344-2347.
8. Rao PV and Gan SH. "Cinnamon: a multifaceted medicinal plant," Evidence-Based Complementary and Alternative Medicine, 2014; 12:2014.

9. Hu XL, Xu Z, and Liu ML. Recent developments of coumarin hybrids as anti-fungal agents. Curr. Top. Med. Chem. 2017; 17:3219–3231.
10. Suleman L. Extracellular Bacterial Proteases in Chronic Wounds: A Potential Therapeutic Target? Advanc. In wound care, 2016; 5(10): 455-463.
11. Ismail SY, Uzairu A. *In silico* QSAR and molecular docking studies of sulfur containing shikonin oxime derivatives as anti-cancer agent for colon cancer. Radiology of infectious diseases, 2019; 6:108-121.
12. Farahpour MR, Amniattalab A, and Hajizadeh H. Evaluation of the wound healing activity of *Cinnamomum zeylanicum* extract on experimentally induced wounds in rats. African J. Biotechn. 2012; 11(84)P: 15068-15071

Table 1. Results of Molecular docking of phytochemicals with alkaline protease.

Mode	Affinity (Kcal/mol)	Distance from rmsd l.b.	Best mode rmsd u.b.
A.	-5.4	0.000	0.000
B.	-5.1	1.967	2.906
C.	-5.0	28.971	30.214
D.	-5.0	2.532	4.477
E.	-4.8	2.212	2.807
F.	-4.8	10.941	13.318
G.	-4.8	12.697	13.847

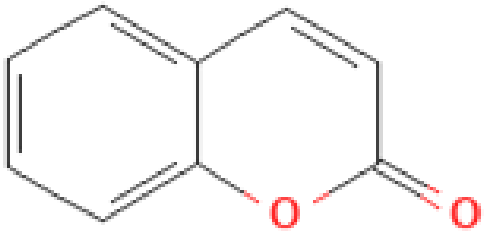

Figure 1: Chemical structure of coumarin

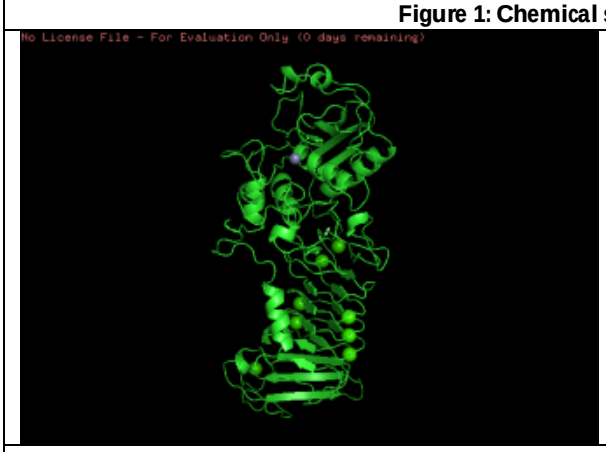

Figure 2. 3D view of alkaline protease from *P.aeruginosa* (PDB ID: 1akl)

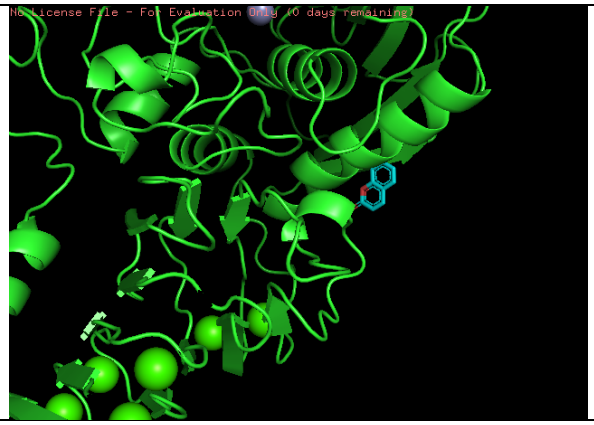

Figure 3. Docking results obtained with AutoDock Vina using PyMOL indicating interaction

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RESEARCH ARTICLE

Effect of Covid-19 Pandemic on Teaching & Learning Process - Students Perspective in India

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ABSTRACT

The neoteric epidemic of the Corona virus pandemic risen the rifts within the education belt worldwide. COVID-19 has terminated educational annoyances, and global health links that revealed very rigid to manage by global health systems. On 11th march 2020Universities around the world had to close their campuses down in the spring of 2020 and shift all their academic program's online. Most of the higher education system is operating through the E- learning with this regard the study tries to analyze the perceptions of teachers and students on the effectiveness of online courses over traditional classroom learning. After two months of online learning, an anonymous questionnaire was sent online to all undergraduate and post graduate students Bagalkot district. All respondents were fully informed about the objectives of the study and agreed to voluntarily participate. A total of 121 students participated in this study. It is concluded that in India, despite gaining immense popularity today, digital technology has still not been embraced by the college students for use in teaching. Students are still more leaning towards face to face teaching rather than online teaching. The institution and faculty members should take necessary measures for improving online teaching quality to help with better learning of students during the COVID-19 era.

Keywords: COVID-19, Online teaching, Higher education and Chi-square test

INTRODUCTION

The neoteric epidemic of the Corona virus pandemic risen the rifts within the education belt worldwide. COVID-19 has terminated educational annoyances, and global health links that revealed very rigid to manage by global health

systems. The corona virus pandemic has no boundaries, and therefore the effect is large and fast. Just within a few months of the outbreak of the disease, it has drastically changed the lifestyle of the whole world with billion of individual being forced to 'stay at home', 'observe self isolations', and work and learn from home. The corona virus disease 2019(covid-19) was detected in china in December 2019. Spread throughout the world within a few months and was declared a pandemic by the world health organization. On 11th march 2020. Universities around the world had to close their campuses down in the spring of 2020 and shift all their academic program's online. Most of the higher education system is operating through the E- learning. To tackle the covid-19 pandemic all most all the world the education ministry has issued the ordered to close the public school and higher education closure as an emergency measure to stop spreading and inflection. Technologies have changed the tradition way of education to the modern way of learning like artificial intelligence (Di Vaio et al 2020) thus E-learning is covered under a larger term of technology based learning through websites learning portals video conferencing YouTube mobile apps, thousands types of free available websites for blended learning tools. Currently E-learning is enhancing student's knowledge even the academic staff and professionals it Adams et al 2018 and Chopra et al 2019).

In India due to covid-19 out brake universities closed and lock down most teachers and students are happy by the move online education. Regardless the country has been revamping to the new-age learning, but there still lounge an hindrance in achieving complete success as only 45 crore people of our total population of the country have approach towards the internet/e-learning. The people residing in rural areas are still considerably bereft of the technologies and thus hampering the explanation for online education. The Covid-19 pandemic taught the whole society on how necessity is that the mother of invention by allowing educational institutions and the students to adopt online learning and introduce a virtual learning culture. The pandemic has been driving the education sector ahead with technological revolution and expansion. Thus, COVID-19 pandemic has significantly disrupted the higher education sector and has created many challenges and opportunities for the tutorial institutes to strengthen their technological knowledge and infrastructure and the students to shift for oneself in the vicinity of technological elevation. In this scenario the role played by teachers and students gains due importance as it s their perceptions and attitude which is critical to motivation and learning ultimately is the acceptance of students and teachers that helps in reaping the benefits of online classes with this regard the study tries to analyze the perceptions of teachers and students on the effectiveness of online courses over traditional classroom learning.

METHODOLOGY

On March 23rd, 2020, the Indian government declared a nationwide lockdown, which resulted in the suspension of face-to-face learning in Schools and colleges. All education Institutes were obliged to conduct solely e-learning. After two months of online learning, an anonymous questionnaire was sent online to all undergraduate and post graduate students Bagalkot district. There were no exclusion criteria, each and every student was allowed to complete the questionnaire once. All respondents were fully informed about the objectives of the study and agreed to voluntarily participate. A total of 121 students participated in this study.

RESULT

A total of 121 Graduates Post Graduate students participated in the study. The demographics of the participants are shown in Table-1. It was found that majority of the students age was 18-25 years. There were almost three-fourth of the students were female. It was also found that more than 50% of the participants were belongs to Commerce background and almost 40% were belongs to science background. In terms of educational qualification 71.9% participants were pursuing their graduates and 28.1% participants were pursuing their post-graduation. Table 2 shows that tools used for teaching during COVID-19 to complete the syllabus. Most of the participants said that whatsapp was used for teaching during COVID-19 to complete the syllabus, followed by Google Class room and youtube. Student's perception towards online teaching during COVIDE-19 is given in Table 3. Almost 30% students said that the online teaching was not effective; around 20% students said that it was effective and 9.1% students said

that the online teaching was very effective. Most of the students were disagreeing that the online teaching have benefited for examination purpose. More than three-fourth of the students were worried about their future education. More than 50% students were disagreeing regarding government decision about using online teaching in future is correct and around 30% students were natural about the government decision. The table 4 reveals the difficulties faced while using the online education tool. More than 30% students said that they had faced poor connection problem, followed by lack of internet facility, time management and Voice/Audio not clear. The table 5 shows the advantages and disadvantages of online teaching over traditional teaching. More than 30% students said that they can be attended online class form anywhere is the advantage and less clearness of the concept is disadvantage of the online class.

Overall, 57 students were agreeing that online teaching will have benefit for examination purpose. Out off 57 (47.1%) students 37 (42.5%) were from graduate curriculum and 20 (58.8 %) were from Post-Graduate curriculum. The result of Chi-Square test reveals that there is no significant difference in the opinion between graduate and postgraduate students (p value =0.115). More than three-fourth of the gradate as well as post graduate students were worried about their future education. The result of Chi-Square test reveals that there is no significant difference in the opinion between graduate and postgraduate students (p value =0.718). Overall, 23 students were agreeing that government decision about using online teaching in future is correct. Out off 23 (19.1%) students 19 (21.8%) were from graduate curriculum and only 4 (11.7%) were from Post-Graduate curriculum. The result of Chi-Square test reveals that there is no significant difference in the opinion between graduate and postgraduate students (p value =0.412).

DISCUSSION

Transitioning to online learning due to COVID-19 has been a highly complex undertaking for higher education institutions. The main purpose of this study was to examine the preference and perception of students regarding the online classes. The results of this study showed that students prefer face-to-face instruction over online education. The Same online education. The results also showed that the shifting from offline to online learning was an unpleasant experience and they expressed a negative attitude towards online learning. They not only considered online learning more difficult but also the lack of supporting resources (Less clearness of the concept, Lack of internet facility , Poor Connectivity, Voice/Audio not clear and less clearness of the concept etc.) was an important challenge during the transition to online learning. The same was observed in (Aguilera-Hermida, 2020, Bączek et al. 2020, Abbasi and Ayoob 2020). Our study results also highlighted that whatsaap was used to complete their syllabus. Muthuprasad T et. al (2020) suggested that it will be better if recorded videos are uploaded in the university website so that the learner can access the videos as per the convenience. The result of our study revealed that online learning is Convenient and flexible to attend. The Tsai & Lin (2004); Peng, et al. (2006); was said that Convenience and flexibility were identified as the strength of online classes. Petrides (2002) was also claimed that participants indicated that the online learning was convenient to work with collaborative groups without rearranging the schedule for everyone as one would do in traditional classroom learning. Poole (2000) found that learners often accessed resources for the course from their home computers, the most convenient location for them. Our result also reveals that majority students claimed that government decision about using online teaching in future is not correct. The result of Chi-Square test reveals that there is no significant difference in the opinions between graduate and postgraduate students.

RECOMMENDATIONS

The experience of the faculties may be interconnected with the students' learning experiences. The appropriate content, connectivity, recorded videos along with proper follow up makes online classes on par with the traditional classroom situation. The students and faculties became more knowledgeable of the tools for remote teaching and learning. The attention span during online learning was even shorter than face to face teaching so we recommend that keep the span of online class will shorter than face to face teaching.

CONCLUSION

With endeavours to control the spread of the novel coronavirus, the all the education institutes are shifting from offline to online platforms to catch up with the curriculum. It may be too early to say how students and teachers will deal with online learning as they figure out the limitations, reorient to tackle them but the opinion and willingness of teachers and students is an important consideration which we have tried to document. It is concluded that in India, despite gaining immense popularity today, digital technology has still not been embraced by the college students for use in teaching. Students are still more leaning towards face to face teaching rather than online teaching. The institution and faculty members should take necessary measures for improving online teaching quality to help with better learning of students during the COVID-19 era.

REFERENCES

1. Di Vaio, A., Boccia, F., Landriani, L., Palladino, R.: Artificial intelligence in the agri-food system: Rethinking sustainable business models in the COVID-19 scenario. Sustainability 12(12), 4851 (2020a).
2. Adams, D., Sumintono, B., Mohamed, A., Noor, N.S.M.: E-learning readiness among students of diverse backgrounds in a leading Malaysian higher education institution. Malaysian Journal of Learning and Instruction 15(2), 227–256 (2018).
3. Chopra, G., Madan, P., Jaisingh, P., Bhaskar, P.: Effectiveness of E-learning portal from students' perspective: A structural equation model (SEM) approach. Interactive Technology and Smart Education 16(2), 94–116 (2019). https://doi.org/10.1108/ITSE-05-2018-0027.
4. Abbasi S, Ayoob T, Malik A, Memon SI. Perceptions of students regarding E-learning during Covid-19 at a private medical college. Pak J Med Sci. (36) 2020.
5. Patricia Aguilera-Hermida. College students' use and acceptance of emergency online learning due to COVID-19. International Journal of Educational Research Open. Vol. (1). (2021).
6. Bączek M, Zagańczyk-Bączek M, Szpringer M, Jaroszyński A, Wożakowska-Kapłon B. Students' perception of online learning during the COVID-19 pandemic: a survey study of Polish medical students.
7. Muthuprasad, T., Aiswarya, S., Aditya, K.S., and Jha, G.K., "Students' perception and preference for online education in India DURING Covid-19 Pandemic," Journal of First Look Social Sciences and Humanities Open, 2020. [Online]. Available: http://dx.doi.org/10.2139/ssrn.3596056.
8. Tsai, C.-C., & Lin, C.-C. (2004). Taiwanese adolescents' perceptions and attitudes regarding the Internet: exploring gender differences. Adolescence, 39(159), 725–734.
9. Peng, H., Tsai, C.-C., & Wu, Y.-T. (2006). University students' self-efficacy and their attitudes toward the Internet: the role of students' perceptions of the Internet. Educational Studies, 32(1), 73–86.
10. Petrides, L.A. (2002). Web-based technologies for distributed (or distance) learning: Creating learner-centered educational experiences in the higher education classroom. International Journal of Instructional Media, 29(1), 69-77.
11. Poole, M. Scott, Andrew H. Van de Ven, Kevin Dooley, and M. E. Holmes 2000 Organizational change and innovation processes: Theory and methods for research. New York: Oxford University Press.

Table 1: Demographics of the participants

Age	No. of Participants	Percent
18-25	116	95.9
26-30	2	1.7
More than 30	3	2.5
Gender		
Male	35	28.9
Female	86	71.1

Your stream of education.		
Arts	12	9.9
Commerce	61	50.4
Science	48	39.7
You are studying which of the following curriculum?		
Graduate	87	71.9
Post-Graduate	34	28.1
Total	121	100.0

Table 2: Tools used for teaching during COVID-19 to complete the syllabus.

Options	No. of Participants	Percent
Any other	25	20.7
e-mailing the lectures	2	1.7
Google class room	27	22.3
Whatsapp	38	31.4
Youtube	12	9.9
Zoom app	17	14.0

Table 3: Students perception towards online teaching during COVID-19

How will your rate your experience of online teaching?		
Opinion	**No. of Participants**	**Percent**
Not effective	34	28.1
Less effective	22	18.2
Average	27	22.3
Effective	27	22.3
Very Effective	11	9.1
Online teaching have benefited for examination purpose		
Strongly Agree	3	2.5
Agree	54	44.6
Disagree	49	40.5
Strongly Disagree	15	12.4
Are you worried about your future education?		
Never	2	1.7
No	13	10.7
Somewhat	14	11.6
Yes	92	76.0
Government decision about using online teaching in future is correct		
Strongly Agree	2	1.7
Agree	21	17.4
Neutral	36	29.8
Disagree	44	36.4
Strongly Disagree	18	14.9
Total	121	100.0

Table 4: Difficulties faced while using the online education tool

Opinion	No. of Participants	Percent
Any other	26	21.5
Eye fatigue	6	5.0
Lack of internet facility	22	18.2
Poor Connectivity	37	30.6
Time management	11	9.1
Voice/Audio not clear	19	15.7

Table 5: Advantages and disadvantages of online teaching over traditional teaching

Advantages of online teaching over traditional teaching		
Opinion	**No. of Participants**	**Percent**
Available at any time	24	19.8
Can be attended from anywhere	39	32.2
Easy to understand the concept because of videos	20	16.5
No need to travel	30	24.8
Save time	8	6.6
Disadvantages of online teaching over traditional teaching		
Any other	15	12.4
Attention span is less for online learning	12	9.9
Lectures removed after specified time	1	.8
Less clearness of the concept	40	33.1
Needs internet connectivity	11	9.1
Needs Smartphone/Laptop/Desktop	17	14.0
No interaction with teacher	25	20.7

Table 6: Cross tabulation between benefit of online teaching and Curriculum

Online teaching have benefited for examination purpose	**Curriculum**		**Total**	**p value**
	Graduate	**Post-Graduate**		
Strongly Agree	3	0	3	0.155
	3.4%	0.0%	2.5%	
Agree	34	20	54	
	39.1%	58.8%	44.6%	
Disagree	37	12	49	
	42.5%	35.3%	40.5%	
Strongly Disagree	13	2	15	
	14.9%	5.9%	12.4%	
Are you worried about your future education?				
Never	2	0	2	0.718
	2.3%	0.0%	1.7%	
No	10	3	13	

	11.5%	8.8%	10.7%	
Somewhat	9	5	14	
	10.3%	14.7%	11.6%	
Yes	66	26	92	
	75.9%	76.5%	76.0%	
Government decision about using online teaching in future is correct				
Strongly Agree	1	1	2	
	1.1%	2.9%	1.7%	
Agree	18	3	21	
	20.7%	8.8%	17.4%	
Neutral	23	13	36	
	26.4%	38.2%	29.8%	0.412
Disagree	31	13	44	
	35.6%	38.2%	36.4%	
Strongly Disagree	14	4	18	
	16.1%	11.8%	14.9%	
Total	87	34	121	
	100.0%	100.0%	100.0%	

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RESEARCH ARTICLE

Evaluation of the Nerve Conduction Velocity Declination Rate of Sural Nerve According to age in Healthy People.

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ABSTRACT

The aging process (normal aging) represents a global biological change that occurs with age and is not affected by diseases that have a negative impact on the clinic. Studies have shown that aging affects the anatomy and structural features and function of the peripheral nervous system. There is significant decrease of myelinated and unmyelinated nerve fibers. There is a strong documented effect on the function and electrophysiological properties of peripheral nervous System. Which includes a reduction of nerve conduction velocity, muscle strength discrimination, autonomic responses and endoneurial blood flow. Declinations of conduction velocities may be begin after 30-40 years of age but the values normally change by less than 10m/s at 6th or even in 8th decade. Aim of the study was to evaluate the nerve conduction velocity of sural nerve & to find the declination rate of sural nerve according to age.. 80 healthy subjects having the ages of 30-79 were included in this study, divided into five age groups. Subjects suffer from metabolic, traumatic or nervous disorders affecting the nerves were excluded. Sural Nerve conduction Velocity was evaluated. Significant statistical decrease was found in the nerve conduction velocity of the sural nerve with increasing age, after analyzing the data with SPSS version twenty. The study shows a decrease in the level of nerve velocity of sural nerves (0.37m/s per year). Significant decrease in the sensory conduction velocity line of the sural nerves as age increases.

Keywords: Aging, Sural Nerve, NCV

INTRODUCTION

The aging process (normal aging) refers to universal biological changes that occur with age and are not susceptible to diseases with adverse clinical effects [1]. It has well documented age-related changes occurring in the central and peripheral nervous systems. Studies show that aging affects the anatomy and the structural and functional properties of the peripheral nervous system [2]. Where both myelinated and unmyelinated nerve fibers are lost. There is a well-documented impact on the function and electrophysiological properties of the peripheral nerves. These include nerve conduction velocity, muscle strength discrimination, autonomic responses and deterioration in endoneurial blood flow [2]. The central nervous system, along with the peripheral nervous system, has been implicated in many age-related disorders. Functional deficits may be the result of structural and biochemical changes, resulting in slow loss of neuronal and nerve fibers. This damage is not compensated for as the regeneration and natural capacities of nerve fibers decrease with aging [3] Electrophysiological studies have shown decreasing number of motor units with increasing age, predominantly in the proximal and distal muscles after age 60 [4]. Motor neuron loss is accompanied by a decrease in both the number and diameter of the motor axons. There was an age-related decrease in the number of large and medium myelinated ventral root fibers, but no significant reduction of small nerves fibers. This preference loss is estimated to be 5% from young to old age [5]. However, there are some differences between the reports regarding the rate of decline. Some studies have observed a linear decrease in the speed of nerve conduction with age [3].

MATERIAL AND METHODS

This study was carried after the obtaining Institutional Ethical Clearance certificate form institute. The study included 80 moderately healthy people aged 30–79 years. They are divided into five groups (Group 1: 30–39 Group 2: 40–49, Group: 50–59, Group: 60–69, Group 5:70–79). Subjects were excluded if they were bothered with the report of history of diabetes or any metabolic disorders, neurological disorders, peripheral neuropathy, cognitive impairment, recent surgery. Those who smoked and consumed alcohol in the past 24 hours were not included [6,7,8,9]. Before obtaining the data collection informed consent were taken from the all the subjects. Items that met the inclusion criteria were selected and informed of the study protocol. All subjects were considered for demographic data on age, height, weight, gender, dominance, and occupation before the procedure. The nerve conduction velocity test was performed only on the dominant limb only. The test protocol was explained to all the subjects. Height (cm) and weight (kg) were recorded using a stature meter and weight machine. Skin temperature (°c) was recorded by an infrared thermometer. According to the protocol, the body parts were cleaned with spirit before the Nerve Conduction Velocity test. Ground electrode, recording electrodes and stimulating electrodes were used to record the sensory nerve conduction velocity. The conduction distance is measured using flexible measuring tape.
Neural conduction velocity (m/s) was measured.

Using the following formula.
Sensory nerve conduction velocity (SNV) = D /L
Where: -D = distance in cm and L = delay in ms.[6,7,8]

Sural Nerve Conduction

The subject was in prone position with a pillow at the ankle joint. Nerve conduction of sural nerve was recorded by placing the surface electrode between lateral malleolus and tendoachillies. The nerve was stimulated antidromically 10-16cm proximal to the recording electrode, distal to lower border of gastronomies' at the junction of middle and lower third of leg. The conduction distance between the recording and stimulating electrode was measured by using measuring tape [6,7,8]. The data was recorded with the use of RMS EMG.EP MARK II (Recorder and Medicare System). Data was analyzed on statistical software SPSS Version twenty.

RESULT

Declination rate of Sural Nerve (SNC) is 0.37 m/s per year.
The results of this study showed that with increasing age, the sensory nerve conduction velocity of the sural nerve (P <0.001) gradually decreased.

DISCUSSION

Nerve Conduction studies are an important method to be used as a diagnostic tool in clinical practice and have been fully validate [10]. There are a number of published studies and reviews on nerve conduction. These include factors affecting nerve velocities. These factors can be divided into biological factors (age, height, sex) and physiological factors (body temperature, study technique), which are related to the physical state of the nerves and muscles [10]. The focus of this study is the effect of age on neural conduction velocity. For the current research it has been observed that Declination rate of Sural Nerve (SNC) is 0.37 m/s per year. The decrease in average nerve conduction velocity in older subjects is explained by a following factors [10, 11,12,13,14,15,16,17,18]

1. Local ischemia due to vascular changes.
2. Metabolic depression associated with changes in permeability or trans-membrane transfer mechanisms of nerve fibers.
3. Selective degradation of fast moving fibers [19].
4. Axon diameter reduction compared to fiber diameter.
5. Axon shape harness.

a. Morphological changes such as loss of myelinated nerve fiber, decrease in size and myelin of the remaining myelinated fibers [20].

There is strong evidence that age and myelin integrity reduces conduction velocity. Along nerve fibers and the age related alteration in structure of myelin conduction velocity [21]. In human peripheral nerves, with age, it has been shown to increase connective tissue and decrease blood vessel endurance. It begins as endothelial proliferation and hybridization of the vessels in the fourth decade of life with an increase in endoperineurium invasion and replacing areas of nerve fibers with connective tissue elements. A gradual decrease in blood supply and an increase in connective tissue leads to a gradual change and a decrease in nerve fibers, especially in the elderly [22]. The present study shows that the sensory nerve conduction velocity of the Sural SNC (0.37 m/s per year) is decreasing, much higher than that of the other nerves. The sural nerve is a purely cutaneous nerve formed by variable contributions from the tibial and peroneal nerve within the popliteal fossa. It runs a superficial course in the posterior leg. The Sural nerve injuries in athletes are very uncommon mainly in Hocky Player, Runner and Football Player etc. It occurs due to injury to direct or indirect trauma to posterior calf muscles with leads to sural nerve entrapment [23,24,25].

CONCLUSION

As increasing age of adults, there is a significant decrease in the conduction velocity of the sensory fibers of the sural nerve. This finding may be useful to confirm the diagnosis of peripheral neurological diseases or any entrapment neuropathy with clinical correlation.

REFERENCES

1. Alexandre Kalache and Bruno Lunenfeld; Men, aging and health.
2. R.Kyle Hanks, Ricardo Pietroban Karen C. Nielsen et al ; The effect of age on sciatic Nerve block duration; international Anesthesia research society 2006; 102; 588-92.

3. Enrique Vedu, Dolares Cebllas, Jarges J. Vilches et al ; Influence of ageing on Peripheral nerve function and regeneration; journal of the peripheral nervousSystems; 191-208(2000)
4. Andrew A. Guccione, Geriatric Physical therapy 2nd edition.7
5. Ashok Kumar and Thomas C. Faster neurophysiology of old neurons and synapses brain aging models, methods and synapses brain aging models , methods andmechanism.9
6. U.K. Mishra J. Kalita clinical neurophysiology; nerve conduction electromyography and evoked potentials.
7. Jun Kimura, Electrodiagnosis in diseases of nerve and muscle principles and practice2nd edition.
8. Michael J Aminoff; Electrodiagnosis in clinical neurology 5th edition.
9. Mohamed Saufi Awang, Jafri Malin Abdullah; Nerve conduction study among healthy Malays, the influence of age, height and body mass index on median, ulnar, commonperoneal and sural nerves ; Malaysian journal of medical sciences vol13 num2, 2006pp 19-23.
10. Dr. Haider Zaigham Baqai, Dr. Muhammad Tariq et al; Sural nerve conduction Velocity; age related variation in our normal population. The professional vol.08,No.04 Oct, Nov, Dec, 2001.
11. DiaShehab et al; Normal values of lower limb nerve conduction in Kuwait; 1999;8: 134-137
12. Mark B. Bromberg et al; Symmetry of normal motor and sensory nerve conduction Measurements. Muscle and Nerves 21; 498-503, 1998.
13. William J. Hennessey, Frank J. E. Falco; Median & ulnar nerve conduction studies: Normative data for young adults, Arch Phys Med Rehabilvol 75 March 1994.
14. P. Bouche, F. cattrlin; Clinical & electrophysiological nervous study of the peripheralnervous system in the elderly; J. Neurol (1993) 240:263-268.
15. Stetson DS, Albers JW, Silverstain BA et al; Effects of age, sex and arthrophometric factors on nerve conduction measures. Muscle Nerve,1992; 15: 1095–104.
16. Heikki A. Lang, AskoPussa; Evoluation of nerve conduction velocity in laterchildhood & adolescence; Muscle & Nerve 8:38-43 January 1985.
17. Konishi H; Motor nerve conduction studies in median and ulnar nerve in old adults over 80 years of age. Nippon SeikeigekaGakkaiZasshi, 1982; 56(4): 305–20.
18. Dorfman LJ. Bosley TM; age-related changes in peripheral and central nerve conduction in man; Neurology 1979; 29: 38-44.
19. Carl W. Lafratta; A study of the relationship of motor nerve conduction velocity in the adult to age, sex and handedness: Archives of Physical Medicine & rehab. Aug-1964.
20. Michael H. Chase; Age dependent changes in cat masseter nerve: an electrophysiological& morphological study; Brain research vol 586 Issue 2 July 1992.pp 279-288.
21. Alan Peters; The effect of normal aging on myelin and nerve fibers a review: Journal of Neurocytology31, 581-593 (2000).
22. Harry H. Wilcox; change in nervous system with age; Vol 71 No.12 Dec 1956.
23. Terence Babwah, Sural Nerve Injury in a Footballer Related to Blunt Leg Trauma, Research in Sports Medicine, Volume 20, 2012 - Issue 1,70-73.
24. Brian J. Toy: Conservative Treatment of Bilateral Sural Nerve Entrapment in an Ice Hockey Player, J Athl Train. 1996 Jan;31(1):68-70.
25. Merrilee Zetaruk, Jeff Hyman: Chapter 32 - Leg Injuries, Clinical Sports Medicine, Medical Management and Rehabilitation, 2007, Pages 441-457.

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RESEARCH ARTICLE

Effect of Various Packaging Materials on Sensory Quality of Herbal Edible Coated Pears

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ABSTRACT

The effect of packaging materials was studied on the quality of herbal edible coated pears by organoleptic evaluation at ambient and low temperature, the coated fruits were packed in jute bags, cloth bags, brown paper bags, HDPE (150 gauge or 38 micron) and LDPE (200 gauge or 50 micron). The coated and packaged fruits were stored at ambient temperature whereas coated fruits packed in HDPE and LDPE bags were stored at low temperature. The effect of packaging materials on herbal edible coated pear fruits was evaluated by sensory evaluation in terms of appearance/color, texture, taste and after taste. On the basis of sensory score, the jute bag was reported the best packaging material for packed coated pear at ambient temperature (31±2°C) during storage, however, HDPE was best packaging material at low temperature for coated pear. On the other hand, the best sensory score was revealed in beeswax herbal edible coated packed pears as compared to uncoated at both temperatures i.e. 31±2°C and 4°C. The packed coated fruits were having good quality and longer shelf life than uncoated samples during storage.

Keywords: Fruits & vegetables, Herbal edible coating, Tulsi (*Ocimum sanctum*), Packaging materials and Sensory attributes.

INTRODUCTION

The consumers are giving the preference to fresh fruits and vegetables for their nutritional components in their daily diet. Therefore, the demand of fresh produce is increasing day by day [2]. Pears are rich source of nutrients and healthy bioactive compounds such as carotenoids (flavonols, anthocyanins, kaemferol and isorhamnetin) and phenolic compounds [5]. After harvesting fresh fruits & vegetables cannot replenish carbohydrates or water, the fresh commodities use the stored sugar or starch in the respiration process and stop when reserve food becomes

finished. As a result, aging begins and responsible for the death and decaying of fresh fruits and vegetables [1]. Horticulture produce are highly perishable and because of this rapid deterioration are found during various processes after harvesting from the field therefore fresh produces are to be handled with much care to minimize the post-harvest losses. Pear (*Pyrus pyrifolia ver. Gola*) is climacteric fruit. In the ripening process, some changes are observed in color, firmness, acidity, sugar content, and aroma development [2]. These nutritional properties of the fresh fruits and vegetables are increasing the demand for fresh fruits and vegetables in the market. Recent studies have been reported to prolong the shelf life of pears using edible coatings that are used to improve the handling characteristics of the fruits and vegetables, reporting the ability of this technological strategy to retard changes in oxygen, aromas, moisture and solute transport [2, 6]. Edible coatings are thin layers of material applied to the surface of the fruit and vegetable as an addition to or replacement for the natural protective waxy coating. Traditionally, edible coatings have been used to reduce water loss, but the recent development of formulated edible coatings with a wider range of permeability characteristics has extended the potential application to fresh produce 4. Therefore there is a growing interest in the use of degradable coatings from polysaccharide, protein, and lipid biopolymers. Edible coatings are preferred to enhance the storage or shelf life of fresh produces not only to have good barrier functionalities to gas and water vapour but also to have good sensory properties such as transparency and blend flavour [7]. Nowadays the edible coatings are one of the most useful innovative techniques of preservation which is used in post-harvest industry for increasing the shelf life and quality of horticulture produces [8]. The herbal edible coating is a novel technique for the post-harvest industry, which plays a significant role to minimize the post-harvest losses of fresh fruits and vegetables by extending the storage life. The researchers are highly focused on edible coating due to its demand and importance in post-harvest industry. Herbal edible coatings are made by incorporating aqueous herbal extract in edible coatings. The function of the edible coating can be improved by including herbs such as neem, mint, aloe vera, tulsi, basil, mentha, which act as antioxidants, antimicrobials, colorants, flavors, fortifying nutrients, and spices in edible coating formulation [9]. The herbal edible coatings enhance the shelf life of fresh fruits and vegetables several folds at ambient as well as low temperature. But not many studies have been conducted to study the effect of various packaging material on the herbal edible coated fruits and vegetables [4]. Hence the main objective of this study was to find out the effect of different packaging material on the sensory attributes of herbal edible coated fruits and vegetables so that a suitable packaging material may be recommended for the herbal edible coated fruits and vegetables.

MATERIALS AND METHODS

Raw Materials

The fruits were selected at green–mature stage from Haridwar, Uttarakhand and transported to the Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan. The fresh pears were selected fresh, mature, clean, uniform in shape and size. The selected pears were divided into two groups on the basis of temperature and coating types demonstrated in Table 1a and 1b. TC_1 and TC_2 was uncoated pear at ambient temperature (31±2°C and 70±8% RH) and low temperature (4°C). Each group was containing 10 pears. Pears were washed with water for 5-7 min and air dried at ambient temperature before applying the herbal edible coating. All parameters performed in the department of food and biotechnology at Jayoti Vidyapeeth Women's University.

Packaging Materials

The packaging materials were procured from Jaipur market. The packaging materials used for packaging the herbal edible coated fruits were jute bags, cloth bags, brown paper bags, HDPE (150 gauge or 38 micron) and LDPE (200 gauge or 50 micron).

Development of Herbal Edible Coatings

The aqueous tulsi leaves extract (TLE) was prepared by using Soxhlet apparatus at 78°C, distilled water used as a solvent. The tulsi leaves extract was evaporated and air dried at ambient temperature. The Herbal edible coatings were prepared from tulsi leaves extract (TLE). Herbal edible coatings were prepared by incorporation of aqueous

tulsi leaves extract (Aq-TLE) in four edible coatings separately i.e. chitosan, alginate, cornstarch and beeswax. The addition of Aq-TLE was the same quantity in all herbal edible coatings. The percentage of Aq-TLE was decided after the preliminary study of herbal edible coating applied to fruits. For coating preparation, chitosan, alginate, cornstarch and beeswax were used as a base material in the herbal edible coatings. The chitosan herbal edible coating was prepared by the dissolving the chitosan (1g) in 0.5% acetic acid solution by continuous stirring for 20 min at room temperature then filtered the chitosan coating solution and Aq-TLE (aqueous tulsi leaves extract) and glycerol was added and mixed and stored at room temperature (25-30°C). The alginate herbal edible coating was prepared by dissolving the alginate (2.5 g) in distilled water at 70°C for 10-15 minutes with continuous stirring. Alginate solution was cooled at room temperature (25-30°C) and filtered. Then Aq-TLE (aqueous tulsi leaves extract), glycerol and tween 80 were added in the filtered edible coating. In this coating, the glycerol was used as a plasticizer and tween 80 as a surfactant. The prepared edible coating solution was stored in the refrigerator. Cornstarch herbal edible coating solution was prepared by dissolving 2.5% (w/v) cornstarch and 1.5 g dried tulsi leaves extract (Aq.) in distilled water with agitation for 15 minutes at 90ºC. The pH value was adjusted to 5.6 with 50% (w/v) citric acid solution. Glycerol was added as a plasticizer (2ml/L solution). The beeswax herbal edible coating was prepared by the beeswax (6 g), soy lecithin (10 g) added as an emulsifying agent and aqueous tulsi leaves extract (1.5 g). Beeswax melted at 55-60 °C and mixed with aqueous tulsi leaves extract and 10% soy lecithin solution for 15-20 min with continuous stirring and cool at ambient temperature. Treatments details are given below.

Application of herbal edible coating: The herbal edible coating applied on pears by spraying method and then the residual coating solution was allowed to drip off for a minute. When the pears get dried completely after coating, they were stored at ambient temperature (31±1°C) and low temperature (4°C) for physiochemical analysis.

Sensory Evaluation: The sensory evaluation was performed by using 9 point Hedonic scale, by semi-trained panel members having 10 or 12 panel members. The panel members were provides a 9 point hedonic scale questionnaire to test appearance color, taste, texture, flavor, after taste and overall acceptability of coated pear and control. They were scored on a scale of 1-9 (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5= neither like nor dislike, 6= like slightly, 7= like moderately, 8 = like very much and 9= like extremely).

Statistical Analysis: The mean and standard deviation was determined for the statistical analyses of the data using MS Excel 2007.

RESULTS AND DISCUSSION

Appearance/Color

The effect of packaging material on appearance/color of coated pears is shown in Figure 1 & 2 and data is presented in Table 2a, 2b and 3. Coated pears packed in various packaging materials; the sensory scores for color decreased progressively throughout the storage time at ambient and low temperature and did not show any significant change. The sensory score of appearance for packed coated pears at 0^{th} day of storage was 9.0. On 50^{th} day of storage, the sensory score of appearance for packed coated pears was 6.4 (T_1), 5.8 (T_2), 6.0 (T_3), 5.8 (T_4), 5.5 (T_5), 6.2 (T_6), 5.2 (T_7), 5.2 (T_8), 5.7 (T_9), 5.4 (T_{10}), 6.0 (T_{11}), 5.4 (T_{12}), 5.9 (T_{13}), 6.5 (T_{14}), 5.7 (T_{15}), 6.8 (T_{16}), 5.7 (T_{17}), 5.8 (T_{18}), 6.2 (T_{19}) and 6.3 (T_{20}). These samples were stored at ambient temperature (31±2°C). However, the sensory score of appearance for packed coated pears at low temperature on 75^{th} day was 5.8 (T_{21}), 5.6 (T_{22}), 5.5 (T_{23}), 5.3 (T_{24}), 5.8 (T_{25}), 5.7 (T_{26}), 6.5 (T_{27}) and 5.7 (T_{28}). On the basis of present investigation, the coated pears packed in jute bag found highest appearance score in all samples as compared to other packaging materials at ambient temperature (31±2°C) on 50^{th} day. The maximum sensory score of appearance was reported in HDPE packed coated pears at low temperature on 75^{th} day, however the uncoated pears were not acceptable for evaluation of appearance at that time on low temperature (4°C) as well as ambient temperature (31±2°C). Further, the uncoated pears were discarded on 30^{th} day and 45^{th} day at ambient and low temperature respectively. The highest sensory score of appearance was recorded in packed pear fruits as

compared to uncoated. The coated pears packaged in various packaging materials revealed better appearance or color during storage at ambient temperature as compared to uncoated samples which were stored at same temperature and storage time. The packaging material provides suitable environment for gaseous exchange, decrease the rate of transpiration and moisture loss therefore shelf life of fruits is increased. Bhattarai and Shah [10] observed that the effect of packaging materials on mandarin, the five treatments viz. plastic, newspaper wrapping, jute wrapping and no packaging materials (control) were used and found that the shelf life of all packaged fruits was increased as compared -to control. Moreover, the Taduri *et al*.[11] found the fruits packed in perforated LDPE had better colour development as compared to control fruits and it maintained the better quality after two weeks of storage as compared to other treatments. Similarly, Kaur *et al*. [12] revealed the sensory score was gradually decreased during storage. The fruits was packed in CFB boxes with HDPE liners maintained the higher sensory score (7.8).

Texture

The effect of packaging material on the texture of coated pears demonstrated in Table 2a, 2b and 3. For coated pears packed in various packaging materials, the sensory scores for texture decreased progressively throughout the storage time at ambient temperature and did not show any significant change. The sensory score of texture for packed coated pears at 0th day of storage was 8.8.On 50th day of storage, the sensory score of texture for packed coated pears was 6.3 (T_1), 5.6 (T_2), 6.2 (T_3), 5.9 (T_4), 5.5 (T_5), 5.9 (T_6), 5.3 (T_7), 5.4 (T_8), 5.7 (T_9), 5.4 (T_{10}), 6.4 (T_{11}), 6.2 (T_{12}), 5.4 (T_{13}), 6.1 (T_{14}), 5.5 (T_{15}), 6.5 (T_{16}), 5.8 (T_{17}), 6.0 (T_{18}), 6.5 (T_{19}) and 6.4 (T_{20}). These samples were stored at ambient temperature (31±2℃). However, the sensory score of texture for packed coated pears at low temperature on 75th day was 5.9 (T_{21}), 5.5 (T_{22}), 5.7 (T_{23}), 5.4 (T_{24}), 6.1 (T_{25}), 5.5 (T_{26}), 6.5 (T_{27}) and 6.4 (T_{28}). The sensory score of texture for coated pears packed in jute bag was better as compared to other packaging materials at ambient temperature (31±2℃) on 50th day. The maximum sensory score of texture was reported in HDPE packed coated pears at low temperature on 75th day, however the uncoated pears were not acceptable for evaluation of texture at that time on low temperature (4℃) as well as ambient temperature (31±2℃). Further, the uncoated pears were discarded on 30th day and 45th day at ambient and low temperature respectively. The highest sensory score of texture was recorded in packed fruits and vegetables as compared to uncoated. The coated fruits and vegetables packaged in various packaging materials revealed better texture during storage at ambient temperature as compared to uncoated samples which were stored at same temperature and storage time. Similarly, Kaur et al.[12] revealed that the sensory score for was gradually decreased during storage. The fruits was packed in CFB boxes with HDPE liners maintained the texture of fruits on 75th day of storage. Taduri et al.[11] found that the fruits packed in perforated LDPE had more firmness as compared to control fruits and maintained the better quality after two weeks of storage as compared to other treatments. Prasad et al. 13 concluded that the Cool chamber+Brown paper and Cool chamber+Tissue paper were improved the quality of banana fruits alongwith the nutritional and storage properties and these treatments were also found to be good for 3 days of storage both at ambient and cool chamber conditions respectively.

Taste

The effect of packaging material on the taste of coated pears demonstrated in Table 2a, 2b and 3. For coated pears packed in various packaging materials, the sensory scores for taste decreased progressively throughout the storage time at ambient temperature and did not show any significant ($p>0.05$) change. The sensory score of after taste for packed coated pears at 0th day of storage was 8.7. On 50th day of storage, the sensory score of after taste for packed coated pears was 6.8 (T_1), 5.6 (T_2), 5.2 (T_3), 6.5 (T_4), 5.7 (T_5), 6.5 (T_6), 5.5 (T_7), 5.5 (T_8), 6.3 (T_9), 5.8 (T_{10}), 6.5 (T_{11}), 5.6 (T_{12}), 5.8 (T_{13}), 6.3 (T_{14}), 5.8 (T_{15}), 6.5 (T_{16}), 5.7 (T_{17}), 5.6 (T_{18}), 5.7 (T_{19}) and 5.4 (T_{20}). These samples were stored at ambient temperature (31±2□). However, the sensory score of after taste for packed coated pears at low temperature on 75th day was 5.8 (T_{21}), 5.2 (T_{22}), 5.7 (T_{23}), 5.1 (T_{24}), 5.6 (T_{25}), 6.4 (T_{26}), 6.1 (T_{27}) and 5.9 (T_{28}). The coated pears packed in jute bag were found the highest score for after taste as compared to other packaging materials at ambient temperature (31±2**°**C) on 50th day. The maximum sensory score of after taste was reported in HDPE packed coated pears at low temperature on 75th day, however the uncoated pears were not acceptable for evaluation of after taste at that time on low temperature (4°C) as well as ambient temperature (31±2°C). Further, the uncoated pears were discarded on 30th

day and 45th day at ambient and low temperature respectively. The highest sensory score of after taste was recorded in packed pears as compared to uncoated. The coated pears packaged in various packaging materials revealed better after taste during storage at ambient temperature as compared to uncoated samples. The sensory score of after taste for packed fruits and vegetables were observed better as compared to uncoated samples stored at ambient and low temperature both. Taduri *et al*.[11] also found that the fruits packed in perforated LDPE had better flavour and after taste as compared to control fruits and maintained the better quality after two weeks of storage as compared to other treatments [11].

After Taste

The effect of packaging material on the after taste of coated pears fruits demonstrated in Table 2a, 2b and 3. For coated fruits packed in various packaging materials, the sensory scores for after taste decreased progressively throughout the storage time at ambient temperature. After taste score of packed coated pears was significantly decreased ($p<0.05$). The sensory score of after taste for packed coated pears at 0th day of storage was 6.5 (T_1), 5.5 (T_2), 5.6 (T_3), 5.4 (T_4), 5.9 (T_5), 6.5 (T_6), 5.0 (T_7), 6.3 (T_8), 5.9 (T_9), 5.5 (T_{10}), 6.4 (T_{11}), 5.5 (T_{12}), 6.1 (T_{13}), 5.7 (T_{14}), 5.4 (T_{15}), 6.3 (T_{16}), 5.7 (T_{17}), 5.8 (T_{18}), 6.1 (T_{19}) and 5.5 (T_{20}). These samples were stored at ambient temperature (31±2°C). However, the sensory score of after taste for packed coated pears at low temperature on 75th day was 6.2 (T_{21}), 5.1 (T_{22}), 6.0 (T_{23}), 5.9 (T_{24}), 6.3 (T_{25}), 5.7 (T_{26}), 6.3 (T_{27}) and 6.0 (T_{28}). The coated pears packed in jute bag were found the highest score for after taste as compared to other packaging materials at ambient temperature (31±2°C) on 50th day. The maximum sensory score of after taste was reported in HDPE packed coated pears at low temperature on 75th day, however the uncoated pears were not acceptable for evaluation of after taste at that time on low temperature (4°C) as well as ambient temperature (31±2°C). Further, the uncoated pears were discarded on 30th day and 45th day at ambient and low temperature respectively. The highest sensory score of after taste was recorded in packed pears as compared to uncoated. The coated pears packaged in various packaging materials revealed better after taste during storage at ambient temperature as compared to uncoated samples. The sensory score of after taste for packed fruits were observed better as compared to uncoated samples stored at ambient and low temperature both. Taduri *et al*. [11] also found that the fruits packed in perforated LDPE had better flavour and after taste as compared to control fruits and maintained the better quality after two weeks of storage as compared to other treatments.

Overall Acceptability

The effect of packaging material on the overall acceptability of coated pears demonstrated in Table 2a, 2b and 3. For coated pears packed in various packaging materials, the sensory scores for overall acceptability decreased progressively throughout the storage time at ambient temperature and show significant change during storage at both temperatures. The sensory score of overall acceptability for packed coated pears at 0th day of storage was 8.6. On 50th day of storage, the sensory score of overall acceptability for packed coated pears was 6.1 (T_1), 5.5 (T_2), 5.7 (T_3), 5.7 (T_4), 5.4 (T_5), 6.1 (T_6), 5.4 (T_7), 5.0 (T_8), 6.0 (T_9), 5.6 (T_{10}), 6.0 (T_{11}), 5.5 (T_{12}), 5.1 (T_{13}), 5.4 (T_{14}), 5.9 (T_{15}), 6.5 (T_{16}), 5.9 (T_{17}), 5.4 (T_{18}), 6.3 (T_{19}) and 5.8 (T_{20}). These samples were stored at ambient temperature (31±2°C). However, the sensory score of overall acceptability for packed coated pears at low temperature on 75th day was 5.5 (T_{21}), 6.0 (T_{22}), 5.6 (T_{23}), 5.8 (T_{24}), 5.5 (T_{25}), 6.2 (T_{26}), 6.0 (T_{27}) and 7.3 (T_{28}). The sensory score of overall acceptability for coated pears packed in jute bag was better as compared to other packaging materials at ambient temperature (31±2°C) on 50th day. The maximum sensory score of overall acceptability was reported in LDPE packed coated pears at low temperature on 75th day, however the uncoated pears were not acceptable for evaluation of overall acceptability at that time on low temperature (4°C) as well as ambient temperature (31±2°C). Further, the uncoated pears were discarded on 30th day and 45th day at ambient and low temperature respectively. The highest sensory score of overall acceptability was recorded in packed pears as compared to uncoated. The coated fruits and vegetables packaged in various packaging materials revealed better overall acceptability during storage at ambient temperature as compared to uncoated samples which were stored at same temperature and time. The sensory score of overall acceptability for packed fruits and vegetables were observed better as compared to uncoated samples stored at ambient and low temperature both. Similarly, Kaur *et al*.[12] revealed that the sensory score was gradually decreased during storage. The fruits was packed in CFB boxes with HDPE liners maintained the higher sensory score. Taduri *et al*. [11] found that the fruits

packed in perforated LDPE had better colour development as compared to control fruits and maintained the better quality after two weeks of storage as compared to other treatments. Singh *et al*. [14] also reported that the kinnow fruits packaged with cling film (at 15 micron + wax at 10%) was found better quality on 25th day of storage at ambient temperature as compared with other packaging treatments including LDPE (25 micron), HDPE (15 micron), Polypropylene (25 micron), Shrink film (15 micron) and control (open).

CONCLUSION

The results concluded in the present study that the jute bag was best packaging material for pear with herbal edible coatings (cornstarch and beeswax) at ambient temperature. But the HDPE was best packaging material for pear at low temperature. According to sensory score of packaged coated pears, the best packaging material for all coated pears was jute bag at ambient temperature during storage however HDPE was best packaging material at low temperature for pear. There is a need to find alternative post harvest technology for reducing post harvest losses, thus enhancing the shelf life and maintaining the quality of fresh produces at low cost. The results revealed that the herbal edible coating is a good alternative to enhance the shelf life of the fresh produce.

REFERENCES

1. FAO. 1993. Prevention de pérdidas de alimentos poscosecha: Frutas, hortalizas, raíces y tubérculos. Roma, Organización de las Naciones Unidas para la Agricultura y la Alimentación.
2. Kou, X.-H., Guo, W.-L., Guo, R.-Z., Li, X.-Y. and Xue, Z.-H. 2014. Effects of chitosan, calcium chloride, and pullulan coating treatments on antioxidant activity in pear cv. 'Huang guan' during storage. Food and Bioprocess Technology. **7**,(3)- 671–681.
3. Oliveira, C. M., Alpalh˜ao, A., Neto, C. et al. 2006. Efeito do 1- metilciclopropeno em pera 'Rocha' em diferentes estados de maturac¸˜ao armazenadas em atmosfera normal. in Simp´osio Nacional de Fruticultura.. **1**- 232–238. Actas Portuguesas de Horticultura, Alcobac¸a, Portugal.
4. Raghav, P. K., Agarwal, N. and Saini, M. 2016. Edible coating of fruits and vegetables: a review. International Journal of Scientific Research and Modern Education. **I**(I):188-204.
5. Salta, J., Martins, A., Santos, R. G., Neng, N. R., Nogueira, J. M. F., Justino, J. & Rauter, A. P. 2010. Phenolic composition and antioxidant activity of Rocha pear and other pear cultivars - A comparative study. Journal of Functional Food. **2**: 153-157.
6. Silva, de K., Moraes, Fagundes, C., Melo, M. C., Andreani, P. and Monteiro, A. R. 2012. Conservation of *Williams* pear using edible coating with alginate and carrageenan. Ciencia e Tecnologia de Alimentos. **32**(4)- 679–684.
7. Vargas, M., Pastor, C., Chiralt, A., Mc Clements, D. J. and Martinez, C. G. 2008. Recent advances in edible coatings for fresh and minimally processed fruits. Crit Rev Food Sci Nutr. ***48*:** 496-511.
8. Pavlath, A. E. and Orts, W. 2009. Edible films and coating: Why, What and How? In: edible coating and film for food application, Edited by Embuscado, M. E., Hurber K. C., Springer Sc. Business Media, LIC, pp. 1-24.
9. Pranoto, Y., Rakshit, S. K. and Salokhe, V. M. 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. LWT Food Science and Technology. **38**(8)**:** 859–865.
10. Bhattarai, B. P. and Shah, R. 2017. Effect of Different Packaging Materials on Post-Harvest Status of Mandarin (*Citrus reticuleta* Blanco). J Hortic. **4:** 218. doi: 10.4172/2376-0354.1000218.
11. Taduri, M., Reddy, N. N., Jyothi Lakshmi, N. and joshi, V. 2017. Studies on the effect of packaging materials on shelf life of mango cv. Amrapali. The Pharma Innovation Journal. **6**(7): 54-57.
12. Kaur, K., Dhillon, W. S. and Mahajan, B. V. C. 2013. Effect of different packaging materials and storage intervals on physical and biochemical characteristics of pear. J. Food Sci. Technol. **50** (1):147-152.
13. Prasad, R., Ram, R. B., Kumar, V. and Rajvanshi, S. K. 2015. Study on effect of different packaging materials on shelf life of banana (*Musa paradisiaca* L.) cv. harichal under different conditions.Int.J. Pure App.Biosci.**3** (4):132141.

14. Singh, R., Kumar, S., Kumar, A., Pratap, S., Davinder, Singh, H. and Kumar, A. 2017. Studies of Various Packaging Materials and Waxing on Quality Traits of Kinnow. Int. J. Curr. Microbiol. App. Sci. **6**(8): 1370-1379.

Table 1a: Coated pears packed in different packaging materials stored at ambient temperature (31±2°C).

TC_1	Uncoated
CH-HEC	
T_1	CS-HEC coated pears packed in jute bag
T_2	CS-HEC coated pears packed in cloth bag
T_3	CS-HEC coated pears packed in brown paper bag
T_4	CS-HEC coated pears packed in HDPE bag
T_5	CS-HEC coated pears packed in LDPE bag
AL-HEC	
T_6	AL-HEC coated pears packed in jute bag
T_7	AL -HEC coated pears packed in cloth bag
T_8	AL -HEC coated pears packed in brown paper bag
T_9	AL -HEC coated pears packed in HDPE bag
T_{10}	AL -HEC coated pears packed in LDPE bag
CS-HEC	
T_{11}	CS-HEC coated pears packed in jute bag
T_{12}	CS -HEC coated pears packed in cloth bag
T_{13}	CS -HEC coated pears packed in brown paper bag
T_{14}	CS -HEC coated pears packed in HDPE bag
T_{15}	CS -HEC coated pears packed in LDPE bag
BW-HEC	
T_{16}	BW-HEC coated pears packed in jute bag
T_{17}	BW-HEC coated pears packed in cloth bag
T_{18}	BW-HEC coated pears packed in brown paper bag
T_{19}	BW-HEC coated pears packed in HDPE bag
T_{20}	BW-HEC coated pears packed in LDPE bag

Table 1b: Coated pears packed in different packaging materials stored at low temperature (4°C).

TC_2	Uncoated
T_{21}	CH-HEC coated pears packed in HDPE bag
T_{22}	CH-HEC coated pears packed in LDPE bag
T_{23}	AL-HEC coated pears packed in HDPE bag
T_{24}	AL-HEC coated pears fruits packed in LDPE bag
T_{25}	CS-HEC coated pears packed in HDPE bag
T_{26}	CS-HEC coated pears packed in LDPE bag
T_{27}	BW-HEC coated pears packed in HDPE bag
T_{28}	BW-HEC coated pears fruits packed in LDPE bag

Table 2a: Sensory evaluation of packaged coated fruits and vegetables at ambient temperature (31±2°C).

Quality Attributes	Storage Time (Days)	Uncoated (TC_1)	Treatments											
			CH-HEC			AL-HEC			CS-HEC			BW-HEC		
			T_1	T_2	T_3	T_6	T_7	T_8	T_{11}	T_{12}	T_{13}	T_{16}	T_{17}	T_{18}
Appearance/ Color	0th	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00
	15th	7.6±0.29	8.2±0.29	7.8±1.25	8.0±1.00	7.8±0.76	7.5±0.87	7.1±0.00	8.0±0.50	7.1±0.00	7.4±0.58	8.4±0.58	7.6±0.15	7.5±1.04
	30th	5.2±0.58	7.6±0.15	7.3±1.15	7.1±0.00	7.2±1.04	7.0±0.29	6.5±1.00	7.3±0.58	6.5±0.58	6.8±0.29	7.8±0.76	6.7±1.57	7.1±0.00
	45th	-	7.0±2.75	6.8±2.08	6.5±1.00	6.8±0.29	6.2±0.30	6.3±1.44	6.5±0.58	6.0±0.50	6.2±0.30	7.1±0.29	6.5±1.00	6.7±1.57
	50th	-	**6.4±0.29**	**5.8±0.10**	**6.0±0.87**	**6.2±1.26**	**5.2±0.58**	**5.2±0.00**	**6.0±0.50**	**5.4±2.22**	**5.9±1.05**	**6.8±0.29**	**5.7±1.00**	**5.8±0.29**
Texture	0th	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29
	15th	7.4±0.29	8.0±0.50	6.8±2.08	6.9±0.29	7.2±0.77	6.7±1.57	7.1±0.00	7.1±0.50	7.8±0.76	6.6±0.47	7.8±1.04	6.7±1.57	6.8±0.29
	30th	5.0±0.21	7.0±2.75	6.7±1.39	6.6±0.47	7.0±0.53	6.4±0.29	6.5±1.00	6.9±0.29	7.0±0.50	6.5±0.00	7.1±0.00	6.4±0.29	6.2±0.30
	45th	-	6.5±1.00	6.1±0.29	6.5±0.00	6.8±2.47	6.3±1.44	6.3±1.44	6.6±0.47	6.8±0.29	6.2±0.88	6.5±1.00	6.2±1.07	6.5±0.58
	50th	-	**6.3±0.00**	**5.6±0.58**	**6.2±0.88**	**5.9±0.50**	**5.2±0.58**	**5.4±.50**	**6.4±0.88**	**6.2±0.30**	**5.4±0.77**	**6.5±1.44**	**5.8±0.10**	**6.0±0.50**
Taste	0th	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69
	15th	6.2±2.75	7.2±0.00	6.9±0.30	7.1±0.29	7.3±0.07	6.6±0.79	6.8±2.08	7.0±0.50	6.9±0.68	6.7±1.57	8.3±0.58	7.2±0.00	6.9±0.68
	30th	5.3±0.50	6.9±0.30	6.5±0.58	6.8±2.08	6.6±0.79	6.5±0.29	6.3±1.75	6.8±0.29	6.7±1.39	6.4±0.29	7.3±1.40	6.9±0.30	6.7±1.39
	45th	-	6.2±0.53	6.0±0.50	6.3±1.75	6.5±0.29	6.1±0.29	6.2±1.07	6.2±0.30	6.1±0.29	6.2±1.07	6.8±0.77	6.2±0.53	6.1±0.29
	50th	-	**6.8±0.64**	**5.6±0.50**	**5.2±1.07**	**6.5±0.29**	**5.5±0.58**	**5.5±0.53**	**6.5±0.53**	**5.6±0.58**	**5.8±0.76**	**6.5±0.58**	**5.7±0.64**	**5.6±0.58**
After Taste	0th	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58
	15th	6.5±0.50	7.0±0.50	6.6±0.79	6.8±2.08	7.8±2.08	6.4±0.12	7.1±0.29	7.1±0.29	6.8±0.00	7.1±0.29	7.8±0.76	6.9±0.58	7.2±0.00
	30th	5.2±1.00	6.8±0.29	6.5±0.29	6.7±1.39	7.4±1.39	6.1±0.58	6.7±1.57	6.7±1.57	6.5±0.50	6.7±1.57	7.1±0.00	6.4±0.50	6.9±0.30
	45th	-	6.2±0.30	6.1±0.29	6.1±0.29	6.8±0.29	5.5±0.07	6.4±0.29	6.4±0.29	6.0±0.61	6.4±0.29	6.5±1.00	5.9±0.50	6.2±0.53
	50th	-	**6.5±0.58**	**5.5±0.58**	**5.6±0.58**	**6.5±0.58**	**5.0±0.00**	**6.3±1.44**	**6.4±0.00**	**5.5±0.29**	**6.1±0.00**	**6.3±1.44**	**5.7±1.05**	**5.8±0.64**
Overall acceptability	0th	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58
	15th	6.9±0.00	7.3±0.07	6.5±0.58	7.2±0.00	7.2±0.00	6.9±0.30	6.4±0.12	7.0±0.50	6.8±0.58	6.7±1.39	7.5±0.87	7.0±0.39	6.6±0.50
	30th	5.8±0.29	6.6±0.79	6.0±0.50	6.9±0.30	6.9±0.30	6.2±0.53	6.1±0.58	6.8±0.29	6.5±0.50	6.1±0.29	7.0±0.39	6.6±0.50	6.1±0.53
	45th	-	6.5±0.29	5.8±0.50	6.2±0.53	6.2±0.53	5.7±0.64	5.5±0.07	6.2±0.30	6.2±0.50	5.6±0.58	6.7±0.50	6.1±0.53	5.9±2.30
	50th	-	**6.1±0.29**	**5.5±1.05**	**5.7±0.64**	**6.1±0.64**	**5.4±0.39**	**5.0±0.00**	**6.0±0.50**	**5.5±1.05**	**5.1±0.76**	**6.5±0.58**	**5.9±2.30**	**5.4±0.89**

Table 2b: Sensory evaluation of coated fruits and vegetables packed in HDPE and LDPE bags at ambient temperature (31±2°C).

Quality Attributes	Storage Time (Days)	Treatments							
		CH-HEC		AL-HEC		CS-HEC		BW-HEC	
		T_4	T_5	T_9	T_{10}	T_{14}	T_{15}	T_{19}	T_{20}
Appearance/Color	0th	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00
	15th	7.3±0.29	8.1±0.58	7.2±0.00	6.9±0.30	8.3±0.58	7.2±0.00	8.3±0.07	8.6±0.79
	30th	5.8±0.50	7.5±0.50	6.9±0.30	6.2±0.53	7.3±1.40	6.9±0.30	7.6±0.79	7.5±0.29
	45th	-	6.2±0.50	6.2±0.53	5.7±0.64	6.8±0.77	6.2±0.53	6.5±0.29	6.7±0.29
	50th	-	**5.5±1.05**	**5.7±0.64**	**5.4±0.39**	**6.5±0.58**	**5.7±0.64**	**6.2±0.29**	**6.3±0.58**
Texture	0th	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29
	15th	6.6±0.79	6.6±0.79	7.2±0.00	6.9±0.30	7.3±0.07	6.5±0.58	8.3±0.58	8.2±0.29
	30th	5.9±0.29	6.5±0.29	6.9±0.30	6.2±0.53	6.6±0.79	6.0±0.50	7.3±1.40	7.6±0.15
	45th	-	6.1±0.29	6.2±0.53	5.7±0.64	6.5±0.29	5.8±0.50	6.8±0.77	7.0±2.75
	50th	-	**5.5±0.58**	**5.7±0.64**	**5.4±0.39**	**6.1±0.29**	**5.5±1.05**	**6.5±0.58**	**6.4±0.29**
Taste	0th	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69
	15th	7.3±1.40	7.2±0.00	7.8±1.04	7.7±1.57	7.8±1.04	6.7±1.57	7.2±0.00	6.9±0.30
	30th	5.5±0.58	6.9±0.30	7.1±0.00	6.4±0.29	7.1±0.00	6.4±0.29	6.9±0.30	6.2±0.53
	45th	-	6.2±0.53	6.5±1.00	6.2±1.07	6.5±1.00	6.2±1.07	6.2±0.53	5.7±0.64
	50th	-	**5.7±0.64**	**6.3±1.44**	**5.8±0.10**	**6.3±1.44**	**5.8±0.10**	**5.7±0.64**	**6.4±0.39**
After Taste	0th	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58
	15th	6.5±0.58	7.4±0.58	7.3±0.07	6.6±0.79	7.2±0.00	6.9±0.30	7.3±0.07	6.5±0.58
	30th	5.4±2.22	6.8±0.29	6.6±0.79	6.5±0.29	6.9±0.30	6.2±0.53	6.6±0.79	6.0±0.50
	45th	-	6.2±0.30	6.5±0.29	6.1±0.29	6.2±0.53	5.7±0.64	6.5±0.29	5.8±0.50
	50th	-	**5.9±1.05**	**5.9±0.29**	**5.5±0.58**	**5.7±0.64**	**5.4±0.39**	**6.1±0.29**	**5.5±1.05**
Overall acceptability	0th	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58
	15th	6.9±0.30	6.9±0.30	7.0±0.50	6.9±0.68	7.1±0.00	7.4±0.58	7.8±1.04	6.7±1.57
	30th	5.7±0.64	6.2±0.53	6.8±0.29	6.7±1.39	6.5±0.58	6.8±0.29	7.1±0.00	6.4±0.29
	45th	-	5.7±0.64	6.2±0.30	6.1±0.29	6.0±0.50	6.2±0.30	6.5±1.00	6.2±1.07
	50th	-	**5.4±0.39**	**6.0±0.53**	**5.6±0.58**	**5.4±2.22**	**5.9±1.05**	**6.3±1.44**	**5.8±0.10**

Table 3: Sensory evaluation of coated fruits and vegetables packed in HDPE and LDPE bags at low temperature (4°C).

Quality Attributes	Storage Time (Days)	Uncoated (TC_2)	Treatments							
			CH-HEC		AL-HEC		CS-HEC		BW-HEC	
			T_{21}	T_{22}	T_{23}	T_{24}	T_{25}	T_{26}	T_{27}	T_{28}
Appearance/ Color	0th	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00
	15th	7.5±0.87	8.7±0.29	8.4±0.29	8.4±0.47	8.2±0.58	8.3±0.29	8.2±0.29	8.6±0.29	8.2±0.58
	30th	6.4±0.29	7.3±0.58	8.0±0.50	7.4±0.41	7.3±1.04	7.4±0.29	7.5±0.50	8.1±0.41	7.8±1.04
	45th	5.0±0.29	6.9±0.50	7.4±1.04	6.7±0.29	6.6±1.00	6.5±0.50	6.8±1.04	7.2±0.29	7.0±1.00
	60th	-	6.3±0.29	6.5±0.87	6.2±0.29	5.7±2.75	6.0±1.00	6.5±0.87	6.8±0.29	6.5±2.75
	75th	-	**5.6±3.04**	**5.8±2.00**	**5.5±1.04**	**5.3±1.00**	**5.6±0.68**	**5.8±2.00**	**6.5±1.04**	**5.7±1.00**
Texture	0th	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29	8.8±0.29
	15th	7.0±0.39	8.8±0.29	8.4±0.29	8.4±0.29	8.3±0.50	7.8±0.29	8.0±1.00	8.4±0.41	8.2±0.77
	30th	6.6±0.50	7.5±1.57	7.3±0.29	7.2±0.77	7.8±0.58	7.1±0.76	7.8±0.76	8.2±1.00	7.2±0.72
	45th	5.4±0.89	7.0±1.00	6.9±1.00	6.8±2.59	7.1±0.29	6.7±0.50	6.8±2.47	7.2±1.00	6.7±0.42
	60th	-	6.4±0.87	6.3±0.29	6.1±1.57	6.8±0.77	6.2±1.76	6.2±1.55	6.7±0.29	6.4±0.35
	75th	-	**5.8±1.57**	**5.4±0.77**	**5.5±1.32**	**5.6±0.72**	**5.7±0.29**	**5.3±0.30**	**6.1±0.77**	**5.7±0.29**
Taste	0th	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69	8.8±0.69
	15th	7.8±0.29	7.8±0.29	8.0±1.00	8.4±0.41	7.7±0.77	8.5±0.87	7.5±1.50	8.7±0.29	7.8±0.29
	30th	6.4±0.29	7.0±0.76	7.8±0.76	8.2±1.00	7.1±0.72	7.8±1.04	7.1±0.25	7.6±0.29	7.4±0.77
	45th	5.2±0.50	6.5±0.50	6.8±2.47	7.2±1.00	6.2±0.42	7.2±1.04	6.8±0.25	7.0±0.29	6.7±0.72
	60th	-	6.2±1.76	5.8±1.55	6.8±0.29	5.8±0.35	6.7±2.17	6.4±1.87	6.7±0.29	6.4±1.40
	75th	-	**5.8±0.29**	**5.2±0.30**	**5.7±0.77**	**5.1±0.29**	**5.6±0.29**	**5.9±0.58**	**6.1±0.12**	**5.9±1.75**
After Taste	0th	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58	8.7±0.58
	15th	7.0±1.00	8.2±1.00	7.6±0.42	7.8±1.04	7.3±0.25	7.8±0.29	7.0±2.59	7.8±0.29	7.5±0.50
	30th	6.5±3.04	7.8±0.29	7.2±0.35	6.5±2.17	7.2±1.87	6.7±0.29	6.7±1.57	7.7±0.77	7.2±1.76
	45th	5.2±0.53	7.0±0.77	6.8±0.29	6.4±0.29	6.3±0.58	6.4±0.12	6.5±1.32	7.1±0.72	6.8±0.29
	60th	-	6.7±0.72	6.4±0.58	6.2±1.07	6.0±0.61	6.1±0.58	6.2±0.53	6.4±1.40	6.4±0.30
	75th	-	**6.2±1.40**	**5.1±0.76**	**6.0±0.76**	**5.9±2.30**	**6.3±1.44**	**5.7±0.64**	**6.3±1.75**	**6.0±0.58**
Overall acceptability	0th	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58	8.6±0.58
	15th	7.5±0.29	8.2±0.58	7.8±0.29	8.2±0.76	7.8±0.53	7.7±0.77	8.7±0.58	8.0±1.25	8.0±0.50
	30th	6.5±1.50	7.3±1.40	7.3±1.04	7.2±1.89	7.0±3.04	7.2±0.29	8.0±0.00	7.8±0.76	7.8±0.58
	45th	5.8±1.04	6.8±2.47	7.0±0.29	7.0±1.25	6.7±0.29	6.5±0.00	7.5±0.50	7.5±0.26	7.7±1.04
	60th	-	6.0±1.25	6.8±0.00	6.5±0.29	6.5±0.87	6.3±0.29	7.0±0.29	6.8±0.58	7.8±1.25
	75th	-	**5.5±1.00**	**6.0±0.53**	**5.6±0.77**	**5.8±0.29**	**5.5±0.00**	**6.2±0.50**	**6.0±0.00**	**7.3±1.15**

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REVIEW ARTICLE

Role of Chitosan, Gelatin and Manuka Honey in Management of Wound Healing: Some Recent Developments and Future Prospect

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ABSTRACT

Wound healing is a complicated mechanism involving a variety of causes that have direct impact on the skin barrier's re-establishment. Wound causality is encountered very often in various situations, including combat scenario. Chitosan and its derivatives have attracted great attention due to its properties beneficial application to wound healing. Chitin and Chitosan are desirable for wound care because they offer favourable biological and anti-microbial characteristics and high value potential for wound healing. Several compounds like honey are now being used to help wounds recovery faster. Honey can be classified in different categories depending on its origin, with monofloral honey performing to be extra promising and captivating as an herbal treatment. Manuka honey is a monofloral honey derived from the Manuka tree (Leptospermum Scoparium) because of its biological features, particularly its antibacterial and antioxidant characteristics. The effect of Manuka honey on wound healing as well as its antioxidant activity and other essential biological impacts, are then discussed.

Keywords: Wound healing, chitosan, Gelatin, Manuka honey, Therapeutic role

INTRODUCTION

Wounds are physical injury to the skin and surrounding tissues that may be caused by extreme heat, trauma, toxins or microbial pathogens [1,2]. Wound healing begins immediately after an injury and is divided into many stages,

including hemostasis, inflammation, proliferation, re-epithelialization, and angiogenesis [3]. Wound healing is a complex process affecting a number of factors that have a significant effect on the re-establishment of the skin barrier. Several compounds, such as honey, are used to help wounds recover faster [4]. Wound are caused by physical, chemical, electrical, or microbial insults to the tissue, causing molecular, anatomical, and functional integrity of living tissue to be disrupted [5]. Deep wound causes significant fluid loss and tissues damages, impairing much of the skin's vital functions [6]. Wound infection, which is increases the local tissue damage, is a common complication [7], while systemic inflammatory and immunological responses might lead to a higher predisposition to life threatening sepsis and multi-organ failure [8]. Early and adequate health care is critical in such situations to reduce the injury related mortality rates [9]. Wound are categorized as permanent wounds or acute wounds based on their origin and healing time. Acute wounds are a form of tissue injury that takes 8 to 12 weeks to heal. Mechanical injuries (friction contact between skin and hard surfaces), burns and chemical injuries are the most common forms of acute wounds. When it comes to wound, the temperature of the source and the length of the contact is very difficult factors in determining the severity of the wound. Burn wounds usually necessitate specialist treatment due to the trauma they inflict. Healing is a complicated and tricky system initiated in response to a damage that restores the characteristic and integrity of broken tissue. The management of wounds is of high clinical and socio-economic value. Wound healing occupies an important field of research in modern biomedical sciences. Many traditional as well as advanced wound healing drugs have been tested so far for faster recovery from wound damage. The present review discusses some recent development and future prospect in the field of wound healing [10].

Overview of wound Healing Phases

A Wound is an accidental or disturbances of the skin's normal makeup and physiology. Wounds present clinicians around the world with a serious challenge, frequently leading to disease and death. The ideal wound healing process is classified into four different steps: Hemostasis, inflammation, proliferation, and Maturation Phase [11] and these are also shown in [figure 1].

Hemostatic: This is the first step of wound healing involves constriction of blood vessels, resulting in a decrease of blood flow at the injury site [12]. Platelet's accumulation occurs as a result of this, which aids in the closing of the blood vessel walls. Clotting factors are released as a result of the cleavage of fibrinogen to fibrin, which aids in the forming of a fibrin mesh to form a clot. When a breach in the epithelial barrier is detected, the hemostasis period starts immediately. The first fibrin strand begins to adhere in about sixty seconds [13]

Inflammation: The inflammation stage is activated during the hemostasis phase. The primary function of inflammation is cleansing the wound site removing invasive microorganisms. Inflammation is further divided into two stages. The first stage is characterized by the influx of the neutrophil to the site of the wound and late inflammatory phase involves the migration of macrophages [14].

Re-epithelization: Re-epithelialization is the process of restoring the integrity of the epidermal barrier at wound site [15]. The cells, majorly responsible for this process are the keratinocytes. A wound is not considered to be healed in the absence of the epithelization step [16]. Epithelial cells start migrating from the edge of the wounds a few hours after formation of the wound. When epithelial cells from opposing edges collide, migration is halted [17].

Maturation phase: The Maturation phase is also known as the remodeling phase of final stage of wound healing. It's the point where granulation tissue transforms into scar tissue [18]. It begins after the third week and the changes will continued for years. Within a year, the wound reaches Maximum tensile strength with 70 percent of the tensile strength of the initial tissue [19].

Factor affecting wound healing

Overall wound healing process is also mediated through various localized and systemic factors as described below:

Humidity and oxygenation: It is important to maintain a moist wound condition in order for wounds to heal quickly. Moisture help wound healing during the epithelization process [20]. As a result, occlusive dressings are used to protect wounds to provide them with a moist environment. For wound healing, a high oxygen level is ideal. Partly since oxygen, a necessary component of ATP synthesis facilitates angiogenesis, keratinocyte differentiation and migration, fibroblast proliferation, re-epithelialization, and collagen synthesis both of which help to keep wound free of infection. These steps gradually result in a faster wound contraction rate [21, 22]. The amount of superoxide dismutase in a body is also determined by oxygen [23].

Infection:

Infection has the most negative effect on wound healing [24]. The presences of foreign or necrotic material in the wound environment can increase the risk of infection [25]. When infection is present, up through the surface and creates sore or lesion which required treatment through excellent wound care and perhaps administration of antibiotics.

Age and nutrition: Age related impairment in wound healing is associated with a delay in wound healing due to a delayed inflammatory response [26]. T-cell penetration is delayed, collagen synthesis is decreased, and macrophage activity is reduced. A lack of essential vitamins such as vitamin C, vitamin K, vitamin A, and vitamin E has also been related to slow wound healing. These vitamins are important factors for clotting, collagen synthesis and epithelialization. As a result, wound healing is heavily influenced by one's physical fitness.

Stress: Under Stressful condition, the level of pro-inflammatory cytokines which are critically in inflammatory phase of wound healing, are lowered [27, 28].Stress has also been shown to increase the expressions of glucocorticoids which are believed to regulate differentiation, proliferation, gene transcription and immune cell trafficking.

Current Approaches for Wound Healing

There are commercially available which mainly consist of Chitosan, gelatin/collagen and manuka honey are based;

Chitosan

Chitosan is a biodegradable, non-irritant biocompatible, and non-toxic amino polysaccharide obtained by deacetylation of chitin. Chitin is produced industrially by alkaline hydrolysis of chitin, which is spontaneously or block distributed co-polymer of N-acetyl-glucosamine and N-glucosamine units [29, 30]. Chitosan is currently receiving a great deal of interest for medical and pharmaceutical applications. The fundamental reason for this increased interest undoubtedly due to its intriguing inherent features [31]. Moreover, Chitosan is metabolized by certain human enzymes, especially lysozyme and is considered as biodegradable [32]. Biodegradability and biocompatibility, as well as intricate interactions with extracellular matrix components and growth factors, have boosted its usage in tissue engineering applications including skin, bone, and cartilage [33, 34]. A large body of knowledge exists today on the use of Chitosan as safe biomaterials for a variety of applications: there are recent review articles in biomedical sciences and in pharmaceutical sciences [35, 39]. Chitin and Chitosan are useful in wound care because they have Strong biological and antimicrobial qualities, as well as high value lots of potential for wound healing. A vast number research organizations have been working on developing a new, better wound dressing by synthesizing and altering biocompatible materials in recent years [40,42].

Role of Chitosan for wound healing

Chitosan –based materials, especially wound dressings, have been examined for their antibacterial, anti-inflammatory, and biomedical qualities. Chitosan has been illustrated to be particularly biomaterial for wound recovery due to its antibacterial and anti-inflammatory properties [43]. Viable dressings ought to have properties that are suited for a certain sort of wound whereas being reasonable and causing the slightest sum of distress to patients. Numerous discoveries have been distributed, but careful and steady optimized for a specific sort of wound at a sensible low cost and with least bother to patients. Many effects have been reported, however whole and general

characterization of such substances is required. Although a lot of literature is available on Chitosan and modified Chitosan (blends/composites/derivatives) for wound healing, there are many challenges which need to be explored in process of wound healing. The role of Chitosan has been studied in the following areas.

- Modifications of Chitosan for clinical applications
- Antimicrobial nature of Chitosan
- Anti-inflammatory nature of Chitosan
- Biomedical applications of Chitosan
- Wound healings of Chitosan
- Chitosan with natural polymer blend scaffolds in wound healings
- Chitosan based composite scaffolds in wound healings
- Chitosan based sponges in wound healing
- Chitosan based oil immobilized scaffolds in wound healings
- Chitosan based extract immobilized scaffolds in wound healings.

For researchers working in the fields of biological and pharmaceutical sciences, the above investigations are intended to give insights into the usage of these essential Chitosan-based materials.

Gelatin and collagen

Gelatin is obtained by thermal denaturation or physical and chemical degradation of collagen. As a biomaterial, gelatin has several advantages: it is a natural polymer with no antigenicity, it is completely resorbable in vivo, and its physicochemical properties may be altered[44].The capacity of the Chitosan-gelatin network to be employed in human skin fibroblast, keratinocyte transplantation, and skin regeneration has been documented in certain research[45].The structure shaped when gelatin and Chitosan are combined can influence the spatial dispersion of integrin ligands and polycationic Chitosan interaction with the anionic cell surface. Cell attachment, cellular bioactivity, tissue remodeling, and, eventually the quality of the recovered tissue is all affected by these impacts. It appears, this combination can have impact in attachment anticipation with the same reason, something that other analysts detailed some time recently [46]. Collagen is one of the most therapeutically effective materials for wound healing and skin regeneration. The extracellular matrix's main protein is collagen [47]. Both collagen and gelatin were widely utilized as wound dressings and tissue engineering products for human usage, due to their hemostatic qualities, good biocompatibility, decreased cytotoxicity, low antigenicity, regulated biodegradability and ability to stimulate cellular adhesion and growth [48]. Different type of wound dressing is available on the market addressing all aspect of wound care. The advantages and disadvantages of the products available on the market are presented in [Table: 1]. In several studies, both collagen and gelatin polymers were conditioned as powders, gels, films or scaffolds to provide hemorrhage control in various surgical procedures [55]. Wound exudates were absorbed by porous collagen and gelatin matrices, while a moist environment, and were maintained at the same time, promoting wound healing process [56].

Manuka honey

Honey is a sweet natural substance known for its high nutritional value and health advantages, which include antioxidant, bacteriostatic, anti-inflammatory and antimicrobial properties, as well as wound and sunburn healing effects [57]. This honey is derived from the manuka tree, Leptospermum scoparium, of the Myrtaceae family, grows as a shrub or a small tree in New Zealand and eastern Australia. It's a flowering shrub with white, red or pink flowers [Figure 2][58]. In Traditional medicines several extracts of the manuka tree are used as sedatives and wound-healing remedies. Furthermore, abscesses, surgical wound, traumatic wounds, burns, ulcers of various causes have all been traditionally treated with manuka Honey [59]. The key bioactive chemicals in manuka honey, as well as the processes behind their biological activity, are currently being researched.

Use of Manuka Honey of wound treatment

Honey's use in wound healing has been recognized since ancient times. Honey's antibacterial and antioxidant properties help to keep wounds moist, and its high viscosity acts as a protective barrier against microbial infection. It

has both pro- and anti-inflammatory effects, which is helpful for wound healing [60, 65]. Normal wound healing is a complicated process involving a succession of overlapping processes (coagulation, inflammation, cell proliferation and tissue remodeling) in which the injured tissue is gradually eliminated and replaced with restorative tissues [66]. Even though excessive use of ionic silver has raised concerns about the development of bacterial resistance, current therapeutic products widely used in wound care (silver sulfadiazine (SSD), hydrogel, hydrocolloid and alginate dressings impregnated with silver) are considered useful for limiting bacterial infections, [67, 68]. Natural ingredients, such as honey, aloe Vera, or Curcumin, have a cheap cost and no danger of antimicrobial resistance, which are the main grounds for using them in wound therapy [69].

Honey has been established in conventional medicinal as a registered medicinal device, either incorporated into sterile dressings or sterilized in tubes, despite its long history as a topical wound therapy [70]. A two-fold effect on the inflammatory response might explain the reduction in healing time after honey treatment. Honey for starters, prevents a protracted inflammatory response by inhibiting the formation and proliferation of inflammatory cells at the wound site; second it boosts the production of pro inflammatory cytokine, allowing proper healing and stimulating the proliferation of fibroblasts and epithelial cells [71, 72]. Microorganisms colonising a burn site come from the patient's gastrointestinal and respiratory flora, from endogenous skin or polluted external sources [soil, water, air]. Honey use topically clears wound infection quickly, accelerating wound healing in deep surgically infected wounds that haven't responded to antibiotics and antiseptics [73, 76]. A variety of factors impact honey's antibacterial capabilities, the most important of which are the wound PH, Phenolic chemical, pH of honey, H202, and the osmotic pressure causes by the honey itself [77].

Therapeutic roles

Antioxidant and anti-inflammatory activity

Honey is known to have considerable antioxidant capability, which helps modulate free radical generation and protect cell components from their detrimental effects [78,79]. Manuka honey has a large number of Phenolic compounds, as well as other Phenolic compounds that have been shown to have a considerable ability to reduce free radicals, implying that it has antioxidant capabilities [80,81]. Due to its strong bioactive characteristics, it has frequently been used as the "gold standard'' in numerous studies to evaluate and analyze the antioxidant capacity of various varieties of honey from varied botanical and geographical sources. Wild carrot and Portobello honeys are examples of honeys that are different from acacia. The greatest Phenolic content and antioxidant capacity are seen in Manuka honey [82, 83], obtained, from Germany, Algeria, Saudi Arabia, and Scotland, respectively. Similar results are obtained with Malaysian monofloral honeys and Tualang honey, a Malaysian multi-floral jungle honey [84]. The scavenger action of manuka honey against superoxide anion radicals has also been investigated using electronic paramedical resonances. Antioxidant enzyme activities (such as Catalase) have the high antioxidant capacity of its relevant total Phenolic content may be responsible for these advantages.

The findings imply that manuka honey might be used as an alternative natural supplement to enhance physiological oxidative status. Manuka honey has been shown to have anti-inflammatory properties in animals. Microscopic analysis of wound tissues after adding manuka honey to wounds in animal models revealed an anti-inflammatory impact (decrease in the quantity of white blood cells) [85]. MediHoney anti-inflammatory properties aid wound healing as well. The anti-inflammatory process around the wound is responsible for the quantity of wound exudates. As a result, honey's anti-inflammatory properties minimize edema and exudates, which can aid wound healing. This impact is also helping to alleviate the pain produced by pressure on nerve endings and lowers the quantity of prostaglandin generated during inflammatory response [86]. Honey has been shown to have anti-inflammatory properties in both animal models and clinical settings. Honey's anti-inflammatory properties, as well as its stimulatory effects on granulation and epithelialization, aid in pain and edemareduction [87,88]. It can help to reduce hypertrophic scarring by enabling moist healing. Honey also speeds up the healing process by stimulating the angiogenesis, granulation, and epithelialization.

CONCLUSION

Wounds represent a major challenge regardless of all recent advances in wound healing interventions throughout the world. A thorough knowledge of wound biology is essential to facilitate the development of various strategies for wound care management. Our better understanding of indispensable wound healing process including hemostasis, inflammation, proliferation, re-epithelialization, and angiogenesis of specific wound type such as nutritional optimization, debridement, compression, management of ischemia and infections could lead to suitable wound healing agents and wound care management strategies. Chitosan has been shown to be a potential biomaterial to be used in wound healing due to its anti-inflammatory and antibacterial nature. There's a have to be control the physical properties of recognized frameworks to realize such destinations. In spite of the fact that a parcel of literature is available on Chitosan and modified Chitosan (blends/composites/derivatives) for wound mending, there are numerous challenges which have to be investigated within the prepare of wound healing. Chitin NFs has powerful biological activities and several applications were proposed in the biomedical field. Aside from its main components, manuka honey contains a large number of other constituents in small in trace amounts that have a variety of nutritional and biological effects, including antimicrobial and antioxidant properties.

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REFERENCES

1. Rawat S, Singh R, ThakurP, KaurS, Semwal A.Asian Pacific Journal of Tropical Biomedicine, 2, pp. S1910-S1917, 2012.
2. Boateng, J. and Catanzano, O. Advanced therapeutic dressings for effective wound healing—a review. Journal of pharmaceutical sciences, 104(11), pp.3653-3680, 2015.
3. Enoch, S. and Leaper, D.J. Basic science of wound healing. Surgery (Oxford), 26(2), pp.31-37, 2008.
4. Archana, D., Dutta, J. and Dutta, P.K. Evaluation of Chitosan nano dressing for wound healing: Characterization, in vitro and in vivo studies. International journal of biological macromolecules, 57, pp.193-203, 2013.
5. Schilling J.A. wound healing. Psychological review; 48, pp.374-415.
6. Ramey PI, Barret JP, Herndon DN. Thermal injury. Crit Care Clin 15, pp.333-52, 1999.
7. Cumming, J., Purdue, G.F., Hunt, J.L. and O'Keefe, G.E. Objective estimates of the incidence and consequences of multiple organ dysfunction and sepsis after burn trauma. Journal of Trauma and Acute Care Surgery, 50(3), pp.510-515, 2001
8. Hettiaratchy, S. and Dziewulski, P. Pathophysiology and types of burns. Bmj, 328(7453), pp.1427-1429, 2004.
9. Atiyeh, B.S., Gunn, S.W. and Hayek, S.N. State of the art in burn treatment. World journal of surgery, 29(2), pp.131-148, 2005.
10. Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. Ethnopharmacological approaches to wound healing—exploring Medicinal plants of India. Journal of ethnopharmacology 2007; pp.114:103-13.
11. Gosain, A. and DiPietro, L.A., 2004. Aging and wound healing. World journal of surgery, 28(3), pp.321-326.
12. Pool JG. Normal hemostatic mechanisms: a review. The American journal of medical technology, 43, pp.776-80, 1977.
13. Source: Boundless. "Overview of Hemostasis." Boundless Anatomy and Physiology Boundless,2016Retrieved 4 Aug. 2017 from https://www.boundless.com/physiology/textbooks/boundless-anatomy-andphysiology-textbook/cardiovascular-system-blood- 17/hemostasis168/overview-of-hemostasis-836-8220/
14. Reinke, J.M. and Sorg, H. Wound repair and regeneration. European surgical research, 49(1), pp.35-43, 2012.

15. Raja, S.K., Garcia, M.S. and Isseroff, R.R. Wound re-epithelialization: modulating keratinocyte migration in wound healing. Front Biosci, 12(3), pp.2849-2868, 2007.
16. Sivamani K, Garcia M. S, Isseroff R. R. Frontiers in bioscience: a journal and virtual library,12, pp.2849-2868, 2007.
17. Pastar I. Stojadinovic O, Yin N. C, Ramirez H, Nusbaum A. G, Sawaya A, Tomic-Canic M. Advances in wound care, 3, pp.445-464, 2014.
18. Hunt, T.K., Hopf, H. and Hussain, Z. Physiology of wound healing. Advances in skin & wound care, 13, p.6,2000.
19. Sinno H, Prakash S. Complements and the wound healing cascade: an updated review. Plastic surgery international. 2013;
20. Levenson, S.M., Geever, E.F., Crowley, L.V., Oates III, J.F., Berard, C.W. and Rosen, H. Healing of rat skin wounds. Annals of surgery, 161(2), p.293, 1965.
21. Eaglstein, W.H. Moist wound healing with occlusive dressings: a clinical focus. Dermatologic surgery, 27(2), pp.175-182, 2001.
22. Bishop, A. Role of oxygen in wound healing. Journal of wound care, 17(9), pp.399-402, 2008.
23. Rodriguez, P.G., Felix, F.N., Woodley, D.T. and Shim, E.K. The role of oxygen in wound healing: a review of the literature. Dermatologic surgery, 34(9), pp.1159-1169, 2008.
24. Guo, S.A. and DiPietro, L.A. Factors affecting wound healing. Journal of dental research, 89(3), pp.219-229, 2010.
25. Cohen, B.H., Lewis, L.A. and Resnik, S.S., 1975. Wound healing: a brief review. International journal of dermatology, 14(10), pp.722-726, 1975.
26. Dunphy JE. Surgery in the twenty-first century. Surgery. 1; 75, pp.332-7, 1974.
27. Keylock K. T., Vieira V. J., Wallig M. A., DiPietro L. A., Schrementi M. & Woods J. A. AJP: Regulatory, Integrative and Comparative Physiology, 294, pp; R179-R184,2007
28. Godbout, J.P. and Glaser, R. Stress-induced immune dysregulation: implications for wound healing, infectious disease and cancer. Journal of Neuroimmune Pharmacology, 1(4), pp.421-427, 2006.
29. Boyapati, L. and Wang, H.L. The role of stress in periodontal disease and wound healing. Periodontology 2000, 44(1), pp.195-210, 2007.
30. Khor, E. and Lim, L.Y. Implantable applications of chitin and Chitosan. Biomaterials, 24(13), pp.2339-2349, 2003.
31. Freier, T., Koh, H.S., Kazazian, K. and Shoichet, M.S. Controlling cell adhesion and degradation of Chitosan films by N-acetylation. Biomaterials, 26(29), pp.5872-5878, 2005.
32. Berger, J., Reist, M., Mayer, J.M., Felt, O., Peppas, N.A. and Gurny, R. Structure and interactions in covalently and ionically crosslinked Chitosan hydrogels for biomedical applications. European journal of pharmaceutics and biopharmaceutics, 57(1), pp.19-34, 2004.
33. Muzzarelli, R.A.A. Human enzymatic activities related to the therapeutic administration of chitin derivatives. Cellular and Molecular Life Sciences CMLS, 53(2), pp.131-140,1997.
34. Suh, J.K.F. and Matthew, H.W. Application of Chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials, 21(24), pp.2589-2598, 2000.
35. Drury, J.L. and Mooney, D.J 2003. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials, 24(24), pp.4337-4351, 2003.
36. Park, B.K. and Kim, M.M. Applications of chitin and its derivatives in biological medicine. International journal of molecular sciences, 11(12), pp.5152-5164, 2010.
37. Yang, T.L. Chitin-based materials in tissue engineering: applications in soft tissue and epithelial organ. International journal of molecular sciences, 12(3), pp.1936-1963, 2011.
38. Gupta B, Agarwal R, Alam MS. Textile-based smart wound dressings. Indian J Fibre Textile Res 35, pp.174-187, 2010.
39. Laurienzo, P. Marine polysaccharides in pharmaceutical applications: an overview. Marine drugs, 8(9), pp.2435-2465, 2010.
40. Kean, T. and Thanou, M. Biodegradation, biodistribution and toxicity of Chitosan. Advanced drug delivery reviews, 62(1), pp.3-11,2010.
41. Shibata, H., Shioya, N. and Kuroyanagi, Y. Development of new wound dressing composed of spongy collagen sheet containing dibutyryl cyclic AMP. Journal of Biomaterials Science, Polymer Edition, 8(8), pp.601-621, 1997.

42. Draye, J.P., Delaey, B., Van de Voorde, A., Van Den Bulcke, A., De Reu, B. and Schacht, E. In vitro and in vivo biocompatibility of dextran dialdehyde cross-linked gelatin hydrogel films. Biomaterials, 19(18), pp.1677-1687, 1998.
43. Ulubayram, K., Cakar, A.N., Korkusuz, P., Ertan, C. and Hasirci, N. EGF containing gelatin-based wound dressings. Biomaterials, 22(11), pp.1345-1356, 2001
44. Ahmed, S. and Ikram, S. Chitosan based scaffolds and their applications in wound healing. Achievements in the life sciences, 10(1), pp.27-37, 2016.
45. Breazeale, K. There Goes the Bride: A Snapshot of the Ideal Christian Wife. Women and Christianity, pp.3-20,2008.
46. Mao, J.S., Yin, Y.J. and De Yao, K. The properties of Chitosan–gelatin membranes and scaffolds modified with hyaluronic acid by different methods. Biomaterials, 24(9), pp.1621-1629, 2003.
47. Zhou, X.L., Chen, S.W., Liao, G.D., Shen, Z.J., Zhang, Z.L., Sun, L., Yu, Y.J., Hu, Q.L. and Jin, X.D., 2007. Preventive effect of gelatinizedly-modified Chitosan film on peritoneal adhesion of different types. World Journal of Gastroenterology: WJG, 13(8), p.1262, 2007.
48. Sullivan, M.M., Puolakkainen, P.A., Barker, T.H., Funk, S.E. and Sage, E.H. Altered tissue repair in hevin-null mice: Inhibition of fibroblast migration by a matricellular SPARC homolog. Wound repair and regeneration, 16(2), pp.310-319, 2008.
49. Suarato, G., Bertorelli, R. and Athanassiou, A. Borrowing from nature: biopolymers and biocomposites as smart wound care materials. *Frontiers in* bioengineering and biotechnology, 6, p.137, 2018
50. Veves, A., Sheehan, P. and Pham, H. A randomized controlled trial of Promogran vs standard treatment in the management of diabetic foot ulcers. *Arch surg, 137*(11), pp.822-7, 2002.
51. Donaghue, V.M., Chrzan, J.S., Rosenblum, B.I., Giurini, J.M., Habershaw, G.M. and Veves, A. Evaluation of a Collagen-Alginate Wound Dressing in the Management. Adv. Wound Care, 11, pp.114-118, 1998.
52. Shah, S.V. and Chakravarthy, D. Evaluation of a bovine 100% native collagen for the treatment of chronic wounds. *Journal of Wound, Ostomy and Continence Nursing*, *42*(3), pp.226-234, 2015.
53. Salehi, H., Momeni, M., Ebrahimi, M., Fatemi, M.J., Rahbar, H., Ranjpoor, F., Salehi, A. and Moosavizadeh, F., 2018. Comparing the effect of colactive plus ag dressing versus nitrofurazone and vaseline gauze dressing in the treatment of second-degree burns. Annals of burns and fire disasters, 31(3), p.204, 2018.
54. Newman, M.I., Baratta, L.G. and Swartz, K. Activated, Type I collagen (CellerateRx) and its effectiveness in healing recalcitrant diabetic wounds: a case presentation. *Advances in skin & wound care*, *21*(8), pp.370-374, 2008
55. Schonauer, C., Tessitore, E., Moraci, A., Barbagallo, G. and Albanese, V. The use of local agents: bone wax, gelatin, collagen, oxidized cellulose. *Haemostasis in Spine Surgery*, pp.89-96, 2005
56. Agrawal, P., Soni, S., Mittal, G. and Bhatnagar, A. Role of polymeric biomaterials as wound healing agents. The international journal of lower extremity wounds, 13(3), pp.180-190, 2014.
57. Gorgieva, S. and Kokol, V. Collagen-vs. gelatine- based biomaterials and their biocompatibility: review and perspectives. Biomaterials applications for nanomedicine, 2, pp.17-52, 2011.
58. M Alvarez-Suarez, J., Giampieri, F. and Battino, M. Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases. Current medicinal chemistry, 20(5), pp.621-638, 2013
59. Kato, Y., Umeda, N., Maeda, A., Matsumoto, D., Kitamoto, N. and Kikuzaki, H. Identification of a novel glycoside, leptosin, as a chemical marker of manuka honey. Journal of Agricultural and Food Chemistry, 60(13), pp.3418-3423, 2012.
60. Patel, S. and Cichello, S. Manuka honey: an emerging natural food with medicinal use. Natural products and bioprospecting, 3(4), pp.121-128, 2013.
61. Mandal, M.D. and Mandal, S. Honey: its medicinal property and antibacterial activity. Asian Pacific journal of tropical biomedicine, 1(2), pp.154-160, 2011
62. Tonks, A.J., Cooper, R.A., Jones, K.P., Blair, S., Parton, J. and Tonks, A. Honey stimulates inflammatory cytokine production from monocytes. Cytokine, 21(5), pp.242-247, 2013

63. Majtan, J., Kováčová, E., Bíliková, K. and Šimúth, J. The immunostimulatory effect of the recombinant apalbumin 1–major honeybee royal jelly protein–on TNFα release. International immunopharmacology, 6(2), pp.269-278, 2006.
64. Van den Berg, A.J.J., Van den Worm, E., Quarles van Ufford, H.C., Halkes, S.B.A., Hoekstra, M.J. and Beukelman, C.J. An in vitro examination of the antioxidant and anti-inflammatory properties of buckwheat honey. Journal of wound care, 17(4), pp.172-178,2008
65. Ahmad, A., Khan, R.A. and Mesaik, M.A. Anti-inflammatory effect of natural honey on bovine thrombin-induced oxidative burst in phagocytes. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 23(6), pp.801-808, 2009.
66. Falanga, V. Wound healing and its impairment in the diabetic foot. The Lancet, 366(9498), pp.1736-1743, 2005.
67. Percival, S.L., Woods, E., Nutekpor, M., Bowler, P., Radford, A. and Cochrane, C. Prevalence of silver resistance in bacteria isolated from diabetic foot ulcers and efficacy of silver-containing wound dressings. Ostomy/wound management, 54(3), pp.30-40, 2008.
68. Loh, J.V., Percival, S.L., Woods, E.J., Williams, N.J. and Cochrane, C.A. Silver resistance in MRSA isolated from wound and nasal sources in humans and animals. International Wound Journal, 6(1), pp.32-38, 2009.
69. Davis, S.C. and Perez, R. Cosmeceuticals and natural products: wound healing. Clinics in dermatology, 27(5), pp.502-506, 2009.
70. Jenkins R, Cooper R. Improving antibiotic activity against wound pathogens with manuka honey in vitro. PloS one.7:e45600,2012.
71. Tomblin, V., Ferguson, L.R., Han, D.Y., Murray, P. and Schlothauer, R. Potential pathway of anti-inflammatory effect by New Zealand honeys. International Journal of General Medicine, 7, p.149,2014
72. Visavadia, B.G., Honeysett, J. and Danford, M.H., 2008. Manuka honey dressing: An effective treatment for chronic wound infections. British Journal of Oral and Maxillofacial Surgery, 46(1), pp.55-56,2008.
73. Tan, H.T., Rahman, R.A., Gan, S.H., Halim, A.S., Asma'Hassan, S., Sulaiman, S.A. and Kirnpal-Kaur, B.S. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC* complementary and alternative medicine, *9*(1), pp.1-8,2009
74. Ahmed, A.K.J., Hoekstra, M.J., Hage, J.J. and Karim, R.B. Honey-medicated dressing: transformation of an ancient remedy into modern therapy. Annals of plastic surgery, 50(2), pp.143-148,2003.
75. Shupp, J.W., Nasabzadeh, T.J., Rosenthal, D.S., Jordan, M.H., Fidler, P. and Jeng, J.C. A review of the local pathophysiologic bases of burn wound progression. Journal of burn care & research, 31(6), pp.849-873,2010
76. Nisbet, H.O., Nisbet, C., Yarim, M., Guler, A. and Ozak, A. Effects of three types of honey on cutaneous wound healing. Wounds: a compendium of clinical research and practice, 22(11), pp.275-283,2010
77. Alvarez-Suarez, J.M., Tulipani, S., Romandini, S., Bertoli, E. and Battino, M. Contribution of honey in nutrition and human health: a review. Mediterranean Journal of Nutrition and Metabolism, *3*(1), pp.15-23,2010
78. Henriques, A., Jackson, S., Cooper, R. and Burton, N. Free radical production and quenching in honeys with wound healing potential. Journal of Antimicrobial Chemotherapy, 58(4), pp.773-777,2006
79. Alzahrani, H.A., Boukraâ, L., Yuva Bellik, F.A., Bakhotmah, B.A., Kolayli, S. and Sahin, H. Evaluation of the antioxidant activity of three varieties of honey from different botanical and geographical origins. Global journal of health science, *4*(6), p.191,2012
80. Moniruzzaman, M., Sulaiman, S.A., Khalil, M.I. and Gan, S.H. Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with manuka honey. Chemistry Central Journal, 7(1), pp.1-12,2013
81. Jubri, Z., Rahim, N.B.A. and Aan, G.J. Manuka honey protects middle-aged rats from oxidative damage. Clinics, 68, pp.1446-1454,2013
82. Alzahrani, H.A., Alsabehi, R., Boukraâ, L., Abdellah, F., Bellik, Y. and Bakhotmah, B.A. Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. Molecules, 17(9), pp.10540-10549,2012
83. Schneider, M., Coyle, S., Warnock, M., Gow, I. and Fyfe, L. Anti-microbial activity and composition of manuka and Portobello honey. Phytotherapy Research, 27(8), pp.1162-1168,2013

84. Khalil, M.I., Alam, N., Moniruzzaman, M., Sulaiman, S.A. and Gan, S.H. Phenolic acid composition and antioxidant properties of Malaysian honeys. Journal of food science, 76(6), pp.C921-C928,2011
85. Molan PC. A brief review of honey as a clinical dressing. Primary intention,6(4),pp.148-58,1998
86. Simon, A., Traynor, K., Santos, K., Blaser, G., Bode, U. and Molan, P. Medical honey for wound care—still the 'latest resort'? Evidence-based complementary and alternative medicine, 6(2), pp.165-173,2009
87. Al-Waili, N.S., Salom, K., Butler, G. and Al Ghamdi, A.A. Honey and microbial infections: a review supporting the use of honey for microbial control. Journal of medicinal food, *14*(10), pp.1079-1096,2011
88. Oryan A, Zaker SR. Effects of topical application of honey on cutaneous wound healing in rabbits. Journal of Veterinary Medicine Series A. Feb 12;45(1-10), pp.181-8,1998

Table 1: Several Commercially Available Collagen and Gelatin Based for wound Healing

Composition wt.%	Collagen content	Advantages	Disadvantages	Type of wound	Ref
Bovine collagen, oxidised regenerated cellulose	55	Hemostatic effect	Not to be used in case of third-degree burns, bovine sources	Partial and full thickness wound including: abrasions, bleeding surfaces, dehisced surgical incisions.	49
Collagen, calcium alginate	90	Maintain a moist environment	Requires secondary dressing	Partial and full thickness wounds including: bleeding surfaces, abrasions, 2nd degree burns, diabetic or venous ulcers, pressure injuries, dehisced surgical incisions.	50
Bovine collagen, Manuka honey	88	No extra debridement needed	High fetched bovine source	Fractional and full thickness wounds including: bleeding surfaces, scraped area, burns, weight, venous or diabetic ulcers, dehisced surgical incisions.	51
Collagen, sodium alginate	Not available	Inhibits the activity of MMPs	Requires secondary dressing, bovine source	Partial and full thickness wounds including: abrasions, 1st and 2nd degree burns, pressure, diabetic or venous ulcers, dehisced surgical incisions	52
Collagen	65	Maintain a moist environment	Requires secondary dressing, bovine sources	Fractional and full thickness wounds including: surgical wounds, traumatic wounds, 1st and 2nd degree burns weight, venous stasis, blood vessel or diabetic ulcers	53
Porcine gelatin	100	Hemostatic, resorbable	Non elastic	Bleeding wounds	54

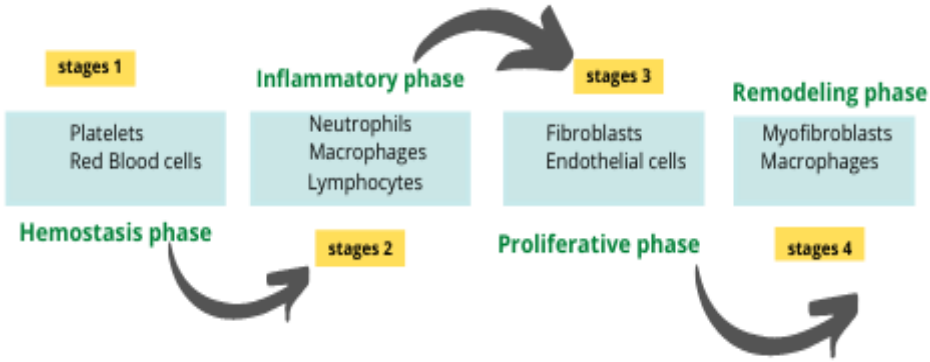

Figure 1: Gelatin and collagen Phases of wound healing

Figure 2: Gelatin and collagen Leptospermum Scoparium plants (manuka honey)

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RESEARCH ARTICLE

Some Properties of Nano $\widehat{\Omega}$-Closed Sets in Nano Topological Spaces

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ABSTRACT

In this paper, we present another class of certain properties of nano $\widehat{\Omega}$-closed sets in nano topological spaces. This class lies between the class of nano closed sets and the class of nano g-closed sets.

Key words and phrases: nano $\widehat{\Omega}$-cld, nano gs-cld, nano sg-cld.

INTRODUCTION

Lellis Thivagar et al [4] presented a nano topological space regarding a subset X of a universe which is characterized as far as lower estimate and upper guess and limit district. The traditional nano topological space depends on an identicalness connection on a set, however in some circumstance, comparability relations are nor appropriate for adapting to granularity, instead the classical nano topology is extend to general binary relation based covering nano topological space. In this paper, we present another class of certain properties of nano $\widehat{\Omega}$-closed sets in nano topological spaces. This class lies between the class of nano closed sets and the class of nano g-closed sets.

PRELIMINARIES

All through this paper $(U, \tau_R(X))$ (or X) address nano topological spaces on which no partition adages are expected to be except if in any case referenced. For a subset H of a space $(U, \tau_R(X))$,N$cl(A)$ and N$int(A)$ signify the nano conclusion of H and the nano inside of H separately. We review the accompanying definitions which are helpful in the continuation.

Definition 2.1.[8] Let U be a non-empty finite set of objects called the universe and R be an equivalence relation on U named as the indiscernibility relation. Elements belonging to the same equivalence class are said to be indiscernible with one another. The pair (U, R) is said to be the approximation space. Let $X \subseteq U$.

(1) The lower approximation of X with respect to R is the set of all objects, which can be for certain classified as X with respect to R and it is denoted by $L_R(X)$. That is, $L_R(X) = \cup_{x \in U} \{R(x): R(x) \subseteq X\}$, where R(x) denotes the equivalence class determined by x.

(2) The upper approximation of X with respect to R is the set of all objects, which can be possibly classified as X with respect to R and it is denoted by $U_R(X)$. That is, $U_R(X) = \cup_{x \in U} \{R(x): R(x) \cap X \neq \phi\}$.

(3) The boundary region of X with respect to R is the set of all objects, which can be classified neither as X nor as not - X with respect to R and it is denoted by $B_R(X)$. That is, $B_R(X) = U_R(X) - L_R(X)$.

Property 2.2.
[4] If (U,R) is an approximation space and X, Y ⊆ U; then

(1) $L_R(X) \subseteq X \subseteq U_R(X)$;
(2) $L_R(\phi) = U_R(\phi) = \phi$ and $L_R(U) = U_R(U) = U$;
(3) $U_R(X \cup Y) = U_R(X) \cup U_R(Y)$;
(4) $U_R(X \cap Y) \subseteq U_R(X) \cap U_R(Y)$;
(5) $L_R(X \cup Y) \supseteq L_R(X) \cup L_R(Y)$;
(6) $L_R(X \cap Y) \subseteq L_R(X) \cap L_R(Y)$;
(7) $L_R(X) \subseteq L_R(Y)$ and $U_R(X) \subseteq U_R(Y)$ whenever $X \subseteq Y$;
(8) $U_R(X^c) = [L_R(X)]^c$ and $L_R(X^c) = [U_R(X)]^c$;
(9) $U_R U_R(X) = L_R U_R(X) = U_R(X)$;
(10) $L_R L_R(X) = U_R L_R(X) = L_R(X)$.

Definition 2.3. [4] Let U be the universe, R be an equivalence relation on U and $\tau_R(X) = \{U, \phi, L_R(X), U_R(X), B_R(X)\}$ where $X \subseteq U$. Then by the Property 2.2, $\tau_R(X)$ satisfies the following axioms:

(1) U and $\phi \in \tau_R(X)$,
(2) The union of the elements of any sub collection of $\tau_R(X)$ is in $\tau_R(X)$,
(3) The intersection of the elements of any finite subcollection of $\tau_R(X)$ is in $\tau_R(X)$.

That is, $\tau_R(X)$ is a topology on U called the nano topology on U with respect to X. We call $(U, \tau_R(X))$ as the nano topological space. The elements of $\tau_R(X)$ are called as nano open sets and $[\tau_R(X)]^c$ is called as the nano closed (briefly, nano cld).

Remark 2.4. [4] If $[\tau_R(X)]$ is the nano topology on U with respect to X, then the set $B = \{U, \phi, L_R(X), B_R(X)\}$ is the basis for $\tau_R(X)$.

Definition 2.5 [4] If $(U, \tau_R(X))$ is a nano topological space with respect to X and if H ⊆ G, then the nano interior of H is defined as the union of all nano open subsets of H and it is denoted by Nint(H).
That is, Nint(H) is the largest nano open subset of H.

The nano closure of H is defined as the intersection of all nano cld sets containing A and it is denoted by Ncl(H). That is, Ncl(H) is the smallest nano cld set containing H.

Definition 2.6

A subset H of a space U is called:

(i) nano semi-open set [4] if H⊆Ncl(Nint(H));

(ii) nano regular open set [4] if H = Nint(Ncl(H)).The complements of the above-mentioned open sets are called their respective closed sets.

Definition 2.5

A subset H of a space Uis called:

(i) a nano generalized closed (briefly,nano g-cld) set [2] if Ncl(H) ⊆G whenever H⊆Gand U is nano open in U.

(ii) a nano semi-generalized closed (briefly,nano sg-cld) set [1] if Nscl(H) ⊆G whenever H⊆G and G is semi-open in U.

(iii) a nano generalized semi-closed (briefly,nano gs-cld) set [1] if Nscl(H) ⊆G whenever H⊆G and G is nano open in U. The complements of the above-mentioned closed sets are called their respective open sets.

Definition 2.6 [3]

A subset H of U is said to be nano locally closed (briefly, nano locally cld) set if H = G∩ F, where G is nano open and F is nano cld in U.

Definition 2.7 [5]

(i) A subset H of U is called a nano $\hat{\Omega}$-closed (briefly, nano $\hat{\Omega}$-cld) set if Ncl(H) ⊆G whenever H⊆G and G is nano gs-open in U.

The collection of all nano $\hat{\Omega}$-closed (resp. nano $\hat{\Omega}$-open) sets is denoted by N$\hat{\Omega}$C(U) (resp. N$\hat{\Omega}$O(U)).

PROPERTIES OF NANO $\hat{\Omega}$-CLOSED SETS

In this section, we discuss some basic properties of nano $\hat{\Omega}$-cld sets.

Definition 3.1

The intersection of all nano gs-open subsets of U containing A is called the nano gs-kernel of H and denoted by Ngs-ker(H).

Lemma 3.2

A subset H of $U$is nano$\hat{\Omega}$-cld if and only if Ncl(H) ⊆Ngs-ker(H).

Proof

Suppose that H is nano $\hat{\Omega}$-cld. Then Ncl(H) ⊆G whenever H⊆G and G is nano gs-open. Let x ∈Ncl(H). If x ∉Ngs-ker(H), then there is a nano gs-open set G containing H such that x ∉G. Since G is a nano gs-open set containing H, we have x ∉Ncl(H) and this is a contradiction.

Conversely, let Ncl(H) ⊆Ngs-ker(H). If G is any nano gs-open set containing H, then Ncl(H) ⊆Ngs-ker(H) ⊆G. Therefore, H is nano$\hat{\Omega}$-cld.

Corollary 3.3

If H is a nano$\hat{\Omega}$-cld set and F is a nano cld set, then H∩ F is a nano $\hat{\Omega}$-cld set.

Proposition 3.4

If H and K are nano$\hat{\Omega}$-cld sets in U, then H∪K is nano $\hat{\Omega}$-cld in U.

Proof

If $H \cup K \subseteq G$ and G is nano gs-open, then $H \subseteq G$ and $K \subseteq G$. Since H and K are nano$\hat{\Omega}$-cld, $G \supseteq Ncl(H)$ and $G \supseteq Ncl(K)$ and hence $G \supseteq Ncl(H) \cup Ncl(K) = Ncl(H \cup K)$. Thus $H \cup K$ is nano $\hat{\Omega}$-cld set in U.

Proposition 3.5

If a set H is nano $\hat{\Omega}$-cld in U, then $Ncl(H) - H$ contains no nonempty nano cld set in U.

Proof

Suppose that H is nano $\hat{\Omega}$-cld. Let F be a nano cld subset of$Ncl(H) - H$. Then $H \subseteq F^c$. But H is nano $\hat{\Omega}$-cld, therefore $Ncl(H) \subseteq F^c$. Consequently, $F \subseteq (Ncl(H))^c$. We already have $F \subseteq Ncl(H)$. Thus $F \subseteq Ncl(H) \cap (Ncl(H))^c$ and F is empty.

The converse of Proposition 3.5 need not be true.

Example 3.6

Let $U = \{a_1, b_2, c_3\}$ with $U/R = \{\{a_1\}, \{b_2, c_3\}\}$ and $X = \{a_1\}$. Then $\tau_R(X) = \{\phi, U, \{a_1\}\}$. Then $N\hat{\Omega}C(U) = \{\phi, \{b_2, c_3\}, U\}$. If $H = \{b_2\}$, then $Ncl(H) - H = \{c_3\}$ does not contain any nonempty nano cld set. But H is not nano$\hat{\Omega}$-cld in U.

Theorem 3.7

A set H is nano $\hat{\Omega}$-cld if and only if $Ncl(A) - A$ contains no nonempty nano gs-cld set.

Proof

Necessity. Suppose that H is nano $\hat{\Omega}$-cld. Let K be a nano gs-cld subset of $Ncl(H) - H$. Then $H \subseteq K^c$. Since H isnano $\hat{\Omega}$-cld, we have $Ncl(H) \subseteq K^c$. Consequently, $K \subseteq (Ncl(H))^c$. Hence, $K \subseteq Ncl(H) \cap (Ncl(H))^c = \phi$. Therefore,K is empty.

Sufficiency. Suppose that $Ncl(H) - H$ contains no nonempty nano gs-cld set. Let $H \subseteq G$ and G be both nano cld and nano sg-open. If $Ncl(H) \not\subset G$, then $Ncl(H) \cap G^c \neq \phi$. Since Ncl(H) is a nano cld set and G^c is both nano open and nano sg-cld set, $Ncl(H) \cap G^c$ is a nonempty nano gs-cld subset of $Ncl(H) - H$. This is a contradiction. Therefore, $Ncl(H) \subseteq G$ and hence H is nano $\hat{\Omega}$-cld.

Proposition 3.8

If H is nano $\hat{\Omega}$-cld in U and $H \subseteq K \subseteq Ncl(H)$, then K is nano $\hat{\Omega}$-cld in U.

Proof

Let $K \subseteq G$ where G is nano gs-open in U. Since $H \subseteq K$, $H \subseteq G$. Since H isnano$\hat{\Omega}$-cld in U, $Ncl(H) \subseteq G$ whenever $H \subseteq G$ and G is nano gs-open in U. Since $K \subseteq Ncl(H)$, $Ncl(K) \subseteq Ncl(H) \subseteq G$. Hence K is nano $\hat{\Omega}$-cld set in U.

Proposition 3.9

If H is a nano gs-open and nano $\hat{\Omega}$-cld in U, then H is nano cld in U.

Proof

Since His nano gs-open and nano $\hat{\Omega}$-cld, $Ncl(H) \subseteq H$ and hence H is nano cld in U.

Recall that a nano topological space Uis called extremally disconnected if Ncl(G) is nano open for each $G \in \tau_R(X)$.

Theorem 3.10

Let Ube extremally disconnected and H is a nano semi-open subset of U. Then H is nano $\hat{\Omega}$-cld if and only if it is nano gs-cld.

Proof

It follows from the fact that if U is extremally disconnected and H is a nano semi-open subset of U, then Nscl(H) = Ncl(H).

Theorem 3.11
Let H be a nano locally cld set of U. Then H is nano cld if and only if H is nano$\widehat{\Omega}$-cld.

Proof
(i) $\Rightarrow$ (ii). It is fact that every nano cld set is nano $\widehat{\Omega}$-cld.
(ii) $\Rightarrow$ (i). We know that, H$\cup$ (U$-$Ncl(H)) is nano open in U, sinceH isnano locally cld. Now H$\cup$ (U$-$Ncl(H)) is nano gs-open set of U such that H$\subseteq$H$\cup$ (U$-$Ncl(H)). Since H isnano $\widehat{\Omega}$-cld, then Ncl(H) $\subset$H$\cup$ (U$-$Ncl(H)). Thus, we have Ncl(H) $\subseteq$H and hence H is a nano cld.

Proposition 3.12
For each x $\in$U, either {x} is nano gs-cld or {x}^{c} is nano $\widehat{\Omega}$-cld in U.

Proof
Suppose that {x} is not nano gs-cld in U. Then {x}^{c} is not nano gs-open and the only nano gs-open set containing {x}^{c} is the space U itself. Therefore Ncl({x}^{c}) $\subseteq$U and so {x}^{c} is nano $\widehat{\Omega}$-cld in U.

Theorem 3.13
Let H be a nano $\widehat{\Omega}$-cld set of a nano topological space U. Then,
(i) Nsint(H) is nano$\widehat{\Omega}$-cld.
(ii) If H is nano regular open, then Npint(H) and Nscl(H) are also nano$\widehat{\Omega}$-cld sets.
(iii) If H is nano regular cld, then Npcl(H) is also nano$\widehat{\Omega}$-cld.

Proof
(i) Since Ncl(Nint(H)) is a nano cld set in U, by Corollary 3.3, Nsint(H) = H$\cap$Ncl(Nint(H)) is nano$\widehat{\Omega}$-cld in U.
(ii) Since H isnano regular open in U, H = Nint(Ncl(H)). Then Nscl(H) = H$\cup$Nint(Ncl(H)) = H. Thus, Nscl(H) is nano $\widehat{\Omega}$-cld in U. Since Npint(H) = H$\cap$Nint(Ncl(H)) = H, Npint(H) is nano $\widehat{\Omega}$-cld.
(iii) Since H isnano regular cld in U, H = Ncl(Nint(H)). Then Npcl(H) = H$\cup$Ncl(Nint(H)) = H. Thus, Npcl(H) is nano $\widehat{\Omega}$-cld in U.

REFERENCES

1. K.Bhuvaneshwari and K. Ezhilarasi, On Nano semigeneralized and Nano generalized semi-closed sets, IJMCAR, 4(3)(2014), 117-124.
2. K.Bhuvaneshwari and K. MythiliGnanapriya, Nano Generalizesdcclosed sets, International Journal of Scientific and Research Publications, 4(5)(2014),1-3.
3. K.Bhuvaneswari and K. MythiliGnanapriya, Nano Generalized locally closed sets and NGLC-Continuous Functions in Nano Topological Spaces,J.Math and its Applications, 4(1-A)(2016), 101-106.
4. M. Lellis Thivagar and Carmel Richard, On Nano forms of weakly open sets, International Journal of Mathematics and Statistics Invention,1(1)(2013), 31-37.
5. A. Pandi, R. Bhavani and R. Umamaheswari,Nano $\widehat{\Omega}$-Closed Sets, Journal of XidianUniversity, 15(5) (2021), 351-356.

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RESEARCH ARTICLE

Prevalence of Hamstring Tightness in Subjects with Acute Non Specific Low Back Pain

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ABSTRACT

Low back pain (LBP) is a most common world wide problem and reported high lifetime prevalence of 84% by World Health Organization (WHO).As hamstring is a biarticular and postural muscle, it has tendency to shorten even under normal situations. As the hamstring have its attachment in pelvis, inflexibility of hamstring can alter the pelvis position and spinal mechanics. Hamstring tightness leads to posterior pelvic tilt and decreases lumbar lordsis which may increase spinal loading and giving rise to LBP. So the aim of the study is to find out prevalence of hamstring tightness in subjects with acute non specific LBP.**:** An observational study was conducted at Physiotherapy department, Civil Hospital Ahmedabad. Convenient sampling was used. 100 subjects of both genders with age group of 18-35,subjects who have not taken any physiotherapy treatment, LBP with no specific pathology and LBP less than 6 weeks were included in the study. 30 subjects of LBP with trauma and specific pathology (e.g., infectious and inflammatory diseases, fracture, tumor and structural deformity), any neurological symptoms involving intervertebral disc herniation and radiculopathy , history of knee injury and knee deformity were excluded. Subjects were assessed for hamstring tightness using the 90º-90º straight leg raising test. Out of 100, 90 subjects were having hamstring tightness and hamstring tightness was absent in 10 subjects. So prevalence of hamstring tightness in subjects with acute non specific low back pain is 90%. Hamstring tightness was found in 57 males and 33 female subjects. Prevalence of Hamstring Tightness in Male subjects was 90.48% and in female subjects 89.19%. No statistical difference found in

hamstring tightness between male and female as p>0.05 which was compared by Unpaired T test. The prevalence of hamstring tightness in subjects with acute non specific LBP is very high in age group 18-35. So inclusion of hamstring flexibility exercise in treatment programs of LBP subjects is very much important.

Key words: Acute non specific low back pain, Hamstring tightness, Straight leg raising test.

INTRODUCTION

Low back pain (LBP) is a most common world wide problem and reported high lifetime prevalence of 84% by World Health Organization (WHO) [1]. By the age of 30, 50% population will have to experience a significant incident of low back pain.[1] It affects more than 50% of general population [2]. Based on the causes LBP is classified as Specific and Non-specific LBP. Specific LBP causes are nerve root compression, vertebral fracture, tumor, infection, inflammatory diseases, spondylolisthesis or spinal stenosis [3]. Non-specific low back pain means low back pain not due to a recognizable and known specific pathology like infectious and inflammatory diseases, tumor, vertebral fracture, osteoporosis, structural deformity and radiculopathy [4] Of all the LBP patients 90% are attributed to Non-specific causes, a disorder which is a health problem of high economic importance [4]. Based on the duration Non-Specific LBP (NS-LBP) is classified Acute (Less than 6 weeks), Subacute (6 weeks – 3 Months) and Chronic (More than 3 Months) [5]. Several risk factors for development of Low Back Pain include increased lumbar lordosis, reduced abdominal muscle length and strength, decreased back extensor muscle endurance, back extensor muscle flexibility, length of illiopsoas, hamstring muscle flexibility, body composition etc [6,7,8,9]. Poor Hamstrings muscular flexibility, Poor abdominal strength and Increased level of physical activity and work related postural stress are considered as risk factors for NSLBP [10].

Anatomically hamstrings muscles arises from the inferomedial impression on the upper part on the ischial tuberosity and inserted on the upper part of the posterior surface of tibia [11]. As hamstring is a biarticular and postural muscle, it has tendency to shorten even under normal situations [12]. At the age of 5 to 6 years, when children start to use prolonged sitting positions in school, hamstrings start to become short. Lack of regular exercise ,limited activity and prolonged sitting posture develop hamstrings tightness in non-specific LBP individuals [13]. Hamstring flexibility gives mechanical advantage for the daily functions but spinal mechanics will be compromised if hamstrings become tight or short [14]. Hamstring tightness leads to posterior pelvic tilt and decreases lumbar lordosis [15]. Hamstring muscles tightness and impaired core muscles strength could reduce pelvic mobility and cause undue strain on the lumbar spine. Hamstring tightness along with core muscle weakness can decrease the lumbar lordotic curve which will multiply spinal loading and consequently Lumbo-Pelvic Rhythm will be altered which may produce more strain on the lower back giving rise to LBP [16,17]. Staton and Purdan found no relationship between hamstrings flexibility and development of low back pain [18]. So the present study investigated the prevalence of hamstring tightness in subjects with acute non specific low back pain.

METHODOLOGY

An observational study was conducted at Physiotherapy department, Civil Hospital Ahmedabad from January 2021 to July 2021. Ethical clearance was taken from the Institutional Ethical Committee. Convenient sampling technique was used. Out of 130 subjects, 100 subjects were included in the study based on inclusion criteria. Informed consent was taken from all subjects. Subjects of both genders with age group 18-35 years, subjects who have not taken any physiotherapy treatment, low back pain with no specific pathology and LBP less than 6 weeks were included in the study. LBP with trauma and specific pathology (e.g, infectious and inflammatory diseases, fracture, tumor and structural deformity), any neurological symptoms involving intervertebral disc herniation and radiculopathy, history

of knee injury and knee deformity subjects were excluded. Subjects were assessed for hamstring tightness using the 90º-90º straight leg raising test [19]. The subjects were in supine position with both hip flexed to 90º degrees and knee flexed. The right lower extremity was tested first and then test was done on the left lower extremity and the pelvis was stabilized to the table with strap. The fulcrum of the goniometer was kept over the lateral condyle of the femur. Proximal arm was kept parallel to the long axis of femur using greater trochanter as a reference. distal arm was kept parallel to the lower leg using the lateral malleolus as a reference. Subject grasps behind the knee with both hands to stabilize hips at 90º of flexion. Then the subject was extend the knee as far as possible. For normal flexibility in the hamstrings knee extension should be within the 20º of full extension. An average of the three repetitions was taken as final reading.

RESULTS

Total 100 subjects with acute non specific low back pain were included in the study. Out of 100, 90 subjects were having hamstring tightness and hamstring tightness was absent in 10 subjects. So prevalence of hamstring tightness in subjects with acute non specific low back pain is 90% (Figure 1). Hamstring tightness was found in 57 males and 33 female subjects (Table 1).Prevalence of Hamstring Tightness in Male subjects was 90.48% and in female subjects 89.19% (Figure 2). Means of Hamstring Tightness in Male and Female subjects were compared with Unpaired t test. No statistical significant difference found in age (p=0.40) between Male and Female (Table 2). No statistical difference found in hamstring tightness on both Right (p=0.81) and Left (p=0.59) side between Male and Female (Table 2). Prevalence of Hamstring tightness is more in mild (20º -25º) tightness group than moderate (26º -30º) and severe (>30º) tightness group (Table 3)

DISCUSSION

The findings of the existing study revealed that ninety percent subjects with acute non-specific LBP had hamstrings tightness. The prevalence of hamstring tightness in male and female subjects were 90.48% and 89.19% respectively. As prevalence of hamstring tightness is very high, it is inferred that hamstring elasticity is compromised in an acute non-specific LBP and hamstring tightness could be one of the causative factors for development of the low back pain. Lower back and back of the thigh are connected by hamstring muscle, alteration in extensibility of hamstring muscle rotate pelvis posteriorly during activities. Due to tight hamstring, alterations in the lumbar curve changes the biomechanical line of pull of back and strains the back during usual day to day activity [20]. So hamstring tightness causes compensatory movement patterns in the lumbar spine which leads to increased stress on the lumbar spine and subsequently increased stress on spinal soft tissues and increased likely hood of injury to the spine. Hence, less hamstring flexibility could be responsible for various postural alterations such as kyphosis of thoracic spine, prolapse intervertebral disc, alteredlumbo-pelvic rhythm and lower back pain, spondylolysis [21].

B.Srinivasan and Pallabi Nanndi(2019) also studied that there is significant difference in hamstring flexibility between non specific LBP patients and matched control group. They found significantly less flexibility in LBP patients. So hamstring stretching exercise is important to include in treatment programs of LBP [22]. Mistry GS, Vyas NJ, Sheth MS (2014) also found that there is a significant difference of hamstrings tightness between the patients having low back pain and healthy individuals [23]. Present study findings correlates well with work done by B. Srinivasan (2019) and Mistry GS(2014). Hamstrings are group of three muscles which are the semi-membranous, semi-tendinous and biceps femoris. The hamstrings are located in the back of the thigh and join the lower pelvis to the lower leg. Hamstrings can flex knee as well as extend hip. The pelvis is connected with spine and other different muscles. These muscles are attached with the spine and help with posture, core stabilization, and spinal movement [11]. If hamstrings become tight then they can restrict pelvis movement. Consequently, limited movement of pelvis can cause the muscles of the low back to become tight which can lead to development of low back pain. Marras WS (1986) also reported that hamstring tightness could be the causative factor of posterior pelvic tilting, reduced lumbar

lordosis and exacerbation of existing pain in patients with low back pain. It has been reported to play a role in different forms of lumbar inter-vertebral disc pathology also [24]. Similarly Bellew *et al* (2010) found that tightness of the hamstrings can affect rotation movement of the pelvic and forward bending range and he concluded that low back pain is strongly correlated with the hamstring flexibility [25]. Koley S, Kaur J, Sandhu JS (2010) have also found poor hamstring flexibility associated with low back pain in young adults [9]. Kamalakannan M, Hemamalini P, Divya T (2020) [26] also concluded that prevalence of hamstring tightness is more among college going students. Their habit of sitting posture with knee flexed for long time could be contributing factor for developing hamstring tightness. Hamstring tightness leads to posterior tilting of pelvis and decrease lordotic curve which can contribute to rise of Mechanical Low back pain. Bhagyashree Koli and Deepak Anap (2018) have studied prevalence of hamstring tightness on age group 18-25 in college going student and found high prevalence of hamstring tightness in college going students. Hence hamstring flexibility program can be used as a preventive tool for musculoskeletal problem of lower back and lower limbs [27].

In disparity to the findings of existing study Shyamal Koley and Neha Likhi (2011) discovered that non-significant negative correlations with hamstring flexibility and LBP [28]. Stutchfield and Coleman (2006) also studied association between hamstring flexibility and low back pain in university male rowers and found no relation between hamstring flexibility and low back pain [29]. Aaron Deline and David Doubblestein (1997) indicated that there may not be significant relationship between hamstring tightness and chronic low back pain in non manual labourers [30]. Present study findings show that there is no significant difference found in age, Hamstring tightness on right side and hamstring tightness on left side between male and female subjects (Table 2). This findings are well correlated with the work done by Dipesh Thakur and Sumi Bose(2016) [31]. Present study findings suggest that prevalence is higher in mild tightness group (20º -25º) compared to moderate (26º -30º) and severe (>30º) tightness group (Table 3). As we have included the subjects who have acute LBP less than 6 weeks, prevalence of hamstring tightness is higher in mild tightness group. If hamstrings tightness is left untreated, LBP can become Chronic[32]and in Chronic LBP patients, it is possible to find more prevalence of hamstring tightness in Moderate and severe tightness group.

The present work findings suggest that we should consider regular hamstring flexibility exercises to prevent LBP and keep our back healthy. Along with the other treatments, Flexibility program also should be included to treat LBP subjects and hamstring flexibility exercise needs to be practiced regularly to prevent LBP. Treatments which includes hamstring flexibility program will add higher value. The hamstrings may become tight due to injury, lack of stretching exercise, prolonged sitting with knee flexed and many other reasons. Lower limb injuries and trauma during sports can develop hamstring tightness. Therefore, sports person should practice regularly hamstring stretching exercise to prevent back injuries and to enhance sports performance. Further studies can be carried out on other contributing factor to develop LBP. Various therapeutic techniques can also be studied in future to treat hamstring tightness in LBP subjects effectively.

CONCLUSION

The prevalence of hamstring tightness in subjects with acute non specific LBP is exceptionally high in 18-35 year age group. So Hamstring tightness is one of the contributing factors to develop non specific LBP. Hence it is important to consider and add hamstring flexibility training in treatment programs of LBP subjects.

REFERENCES

1. Capt. Eric Wilson, Otto Payton, Lisa Donegan-Shoaf, Kartherine Dec. Muscle energy technique in patients with low back pain: A pilot clinical trial. Journal of Orthopaedic and Sports Physical Therapy. 2003; 33(9):502-510.
2. Rozemberg S. Chronic low back pain: Definition and treatment. Rev Prat 2008;58:265-72.

3. Jeannette Saner, Jan Kool, Rob A de Bie, Judith M Sieben and HannuLuomajoki. Movement control exercise versus general exercise to reduce disability in patients with low back pain and movement control impairment. A randomised controlled trial. BMC Musculoskeletal Disorders. 2011; 12:207-213.
4. Federico Balague, Anne F Mannion, FerranPellise, Christine Cedraschi. Non-specific low back pain. Lancet. 2012; 379:482–491.
5. Bogduk N, McGuirk B: Medical Management of Acute and Chronic Low Back Pain: An Evidencebased Approach. Elsevier. 2002; 4(1):33-38.
6. Koley S, Sandhu NK. An association of body composition components with the menopausal status of patients with low back pain in Tarn Taran, Punjab, India. J Life Sci 2009;1:129-132.
7. Koley S, Sharma D. An association of percent body fat with some anthropometric variables in patients with low back pain. South Asian Anthropologist 2009;10:29-33.
8. Koley S, Sharma L, Kaur S. Effects of occupational exposure to whole body vibration in tractor drivers with low back pain in Punjab. Anthropologist. 2010;12:183-187.
9. Koley S, Kaur J, Sandhu JS. Biological risk indicators for non-specific low back pain in young adults of Amritsar, Punjab, India. J Life Sci 2010;2:43-48.
10. Darlene Hertling, Radolph Kessler. Management common musculoskeletal disorders, Physical Therapy Principles and methods. Fourth Edition. 2006; 843-934.
11. Chaurasiya BD. Human anatomy regional and applied. 3rd ed. Vol. 2. New Delhi: CBS Publishers & Distributors; 1998.p.74.
12. Jonhagen S, Nemeth G, Eriksson E. Hamstring injuries in sprinters: the role of concentric and eccentric hamstring muscle strength and flexibility. American Journal of Sports Medicine. 1994; 22:262-266.
13. Foster DN and Fulton MN. Back pain and the exercise prescription. Clinics in Sports Medicine. 1991; 10:187-209.
14. Farfan HF. Muscular mechanism of the lumbar spine and the position of power and efficacy. Orthopaedic Clinics of North American. 1975; 6:135-144.
15. Schafer RC. Clinical Biomechanics: Musculoskeletal actions and reactions. 2nd ed. Baltimore MD: Williams and Wilkins; 1987.
16. Jones MA, Stratton G, Reilly T, Unnithan VB. Biological risk indicators for recurrent non-specific low back pain in adolescents. Br J Sports Med. 2005; 39:137–140.
17. Shannon L. Hoffman, Molly B. Johnson, Dequan Zou, Linda R. Van Dillen. Differences in end-range lumbar

a. flexion during slumped sitting and forward bending between low back pain subgroups and genders. Manual Therapy Journal. 2012; 6:1-7.

18. Staton P, Purdan C. Factors associated with hamstrings injuries: An approach to treatment and preventive measures. J Orthop Sports PhysTher 1989;10:343-349.
19. David J. Magee. Orthopedic Physical Assessment. Fourth Edition. Canada. Saunders Elsevier; 2006. p. 634
20. Negrini S, Giovannoni S, Minozzi S, Barneschi G, Bonaiuti D, Bussotti A, et al. Diagnostic therapeutic flow-charts for low back pain patients: The Italian Clinical Guidelines. EuraMedicophys 2006; 42(2):151-70.
21. O'Sullivan K , Murray E, Sainsbury D. The effect of warm-up, static stretching and dynamic stretching on hamstring flexibility in previously injured subjects. BMC Musculoskeletal Disorders 2009;vol. 10:37.
22. B.Srinivasan, PallabiNanndi.Poor Hamstring Flexibility Causes Non Specific Low Back Pain – A Case Control Study. International journal of basic applied and research 2019:9(7):277-283.
23. Mistry GS, Vyas NJ, Sheth MS. Comparison of hamstrings flexibility in subjects with chronic low back pain versus normal individuals. J ClinExp Res 2014;2:85-88.
24. Marras WS. Flexibility and velocity of the normal and impaired lumbar spine. Archives of Physical Medicine and Rehabilitation 1986; 67: 1865-1869.
25. Bellew S, Ford H, Shere E. The relationship between hamstring flexibility and pelvic rotation around the hip during forward bending. Plymouth Stud J Health Soc Work 2010;2:19-29.
26. Kamalakannan M., Hemamalini P., Divya T. Hamstring tightness causing low back pain among college going students - a cross-sectional study. Biomedicine: 2020; 40(4): 531-534

27. Bhagyashree K. Koli, Deepak B. Anap. Prevalence and severity of hamstring tightness among college student: A cross sectional study. Int. j. clin. biomed. res. 2018;4(2): 65-68.
28. ShyamalKoley, Neha Likhi. No Relationship between Low Back Pain and Hamstring Flexibility Anthropologist, 13(2): 117-120 (2011)
29. Stutchfield BM, Coleman S. The relationships between hamstring flexibility and lumbar flexion and low back pain in rowers. European J Sport Sci 2006;6:255-260.
30. Deline Aaron and Doubblestein David, "The Relationship of Hamstring Length and Chronic Low Back Pain" (1997).Masters Theses.323.
31. Dipesh Thakur, Sumi Rose. A study to find out the correlation between the right and left hamstring length in both gender to determine the prevalence of hamstring tightness among college students. Nitte university journal of health science. 2016; 6(4):46-52.
32. Da Silva Diaz R, Gomez-Conesa A. Shortened hamstring syndrome. Fisioterapia 2005;30:186-193.

Table 1 Hamstring tightness in Male and Female Subjects

Hamstring tightness	Male	Female	Total
Present	57	33	90
Absent	6	4	10
Total	63	37	100

Table 2 Comparison of Age and Hamstring Tightness between Male and Female Subjects

Parameter	Male	Female	p Value	Statistical Significance
Age	27.44±5.63	28.48±5.78	0.4	NS
Hamstring tightness in degree (Right)	25.61º ±4.20º	25.82º ±3.22º	0.81	NS
Hamstring tightness in degree (Left)	24.58º ±4.09º	25.03º ±3.33º	0.59	NS

(NS : Not Significant as p value>0.05)

Table 3 Prevalence of Hamstring Tightness according to severity

Tightness Severity	Male		Female	
	Right	Left	Right	Left
No Tightness(<20)	9.52%	9.52%	10.81%	10.81%
Mild tighness (20-25)	55.55%	63.49%	51.35%	59.46%
Moderate Tightness(26-30)	25.41%	22.23%	35.13%	24.32%
Severe Tightness(>30)	9.52%	4.76%	2.71%	5.41%

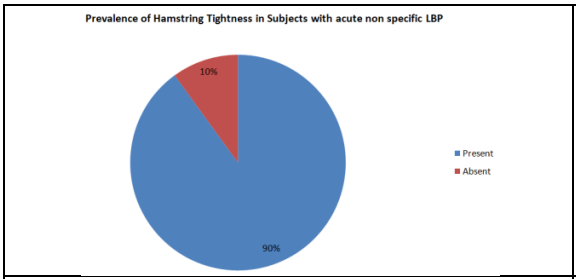

Figure 1. Showing Prevalence of Hamstring Tightness in subjects with acute non specific LBP

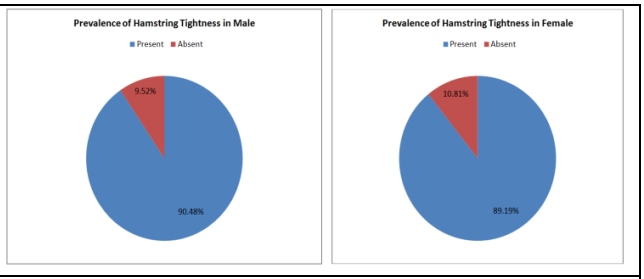

Figure 2. Showing Gender based Prevalence of Hamstring tightness in Subjects with acute non specific LBP

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RESEARCH ARTICLE

Prediction of Antiviral Activity of *Elettaria cardamomum* Targeting SARS-Co-V-2 using Computational Docking Techniques

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ABSTRACT

In December2019, a novel coronavirus, now known as SARS-Co-V-2, emerged as the cause of several acute and atypical respiratory infections at Wuhan, Hubei Province, China. COVID-19 was the name given to the infection caused by this virus. The virus is known to transmit from human-to-human and has caused a worldwide pandemic. SARS-Co-V-2 is highly contagious, and most people in the general community are at risk of infection. The immunological abnormalities caused by SARS-Co-V-2 could lead to microbial infections, pneumonia, gastrointestinal infection, septic shock, and severe multiple organ failure. Due to the serious impact of this virus on the livelihood and downtrading economy of the globe, researchers are exploring several ways to combat its effect, the traditional ways are also reconnoitred. This research focuses on identifying phytochemicals from plant *Elettaria cardamomum* extracts that are capable of fightingCOVID-19. The virus consists of RNA-dependent RNA polymerase enzyme and the interaction between the phytochemical and the viral enzyme was studied employing molecular docking techniques. Molecular docking was carried out by BIOVIA Discovery Studio module of Dassault system. The result analysis and interpretation were based on the –CDOCKER energy and CDOCKER interaction energy. In this study, the high positive value of –CDOCKER energy and –CDOCKER interaction energy implies the high affinity of the phytochemical acetic acid against the RNA-dependent RNA polymerase which efficiently inhibitsits enzymatic metabolic activity.

Keywords: Energy, CDOCKER, phytochemical, coronavirus, RNA

INTRODUCTION

The current covid-19 outbreak is a highly transmissible disease which has its epicentre in the People's Republic of China's Hubei Province. A worldwide health emergency has been proclaimed as a result of rising Case notification

rates in China and around the world by the World Health Organisation in the month of January, 2020(WHO) [1]. Unfortunately, the epidemic disease, was announced by the World Health Organization (WHO) as a pandemic and in accordance of WHO it is the first pandemic that is caused by corona virus [2].As we live in a world which is interconnected on a global scalevia people, goods, food, education, treatment and many other aspects, such type of communications become the most convenient way of viral transmission [3].On getting infected, patients have a maximum chance of developing other symptoms like severe acute respiratory syndrome (SARS), pneumonia, severe symptoms of acute respiratory distress syndrome (ARDS) and in case of high severity condition organ failure may develop [4]. This owes to the novel nature of the virus, and there is a greater uncertainty that surrounds the optimal time for this disease to vanish from the human population [5]. So, prevention in personal level and hygiene is one of the important keys to tackle this pandemic and also helps to break the chain of transmission.

The etiological agent of covid-19 belongs to the family corona viridae. The Corona viridae are a group of plus-stranded RNA viruses that differ in their genetic makeup and in several ways, and also from other plus-strand RNA viruses. Generally, Coronavirus RNA genomes are the largest RNA molecules known in virus, they have helical nucleocapsids, andexpress their plus-stranded RNA genomes using a new approach in which they build a nested set of plus-strand RNA genomes[6, 7].

There are generally four subfamilies of coronaviruses named as alpha, beta, gamma, and delta coronavirus. While alpha- and beta- coronaviruses are thought to have originated in mammals, especially bats, gamma-and delta-coronaviruses are presumed to have originated in pigs and birds. Beta-coronaviruses, can cause significant illness and in cases of severity it can also led to death, whereas alpha-coronaviruses causes asymptomatic or slightly symptomatic infections and are less severe in comparison to beta coronavirus. SARS-Co-V-2 is related to SARS-Co-V-2 and belongs to the B lineage of beta-coronaviruses [8]. The most common type of symptoms associated with SARS is pneumonia and some gastrointestinal complications are found, some asymptomatic stages are found in case of children [9].The lesions caused by SARS-Co-V-2 infection are not limited within the lungs. After entering the body, the virus causes viraemia, which manifests as chills, fever, pharyngalgia, tiredness, diarrhoea, and many other non-specific symptoms [10, 11]. When the virus launches a second time attack, which is also the primary cause of symptom exacerbation,the pulmonary lesions gets worsened at such point, and chest CT scans reveals imaging abnormalities compatible with COVID-19. During such severe condition the number of T and B cells in the peripheral blood decrease dramatically and the inflammatory factors present in the peripheral blood stream increases [12].

As there is no particular molecular action associated with COVID manifestation, therefore therapeutics or drugs are discovered to act against the COVID-19 symptoms based on the regular mutation in the viral genome that is found in different geographical regions. Administration of vaccine, to a greater extent, could be a novel approach to act against this pandemic in a high percentage in comparison to other treatment procedures. Although much about the immune response to SARS-Co-V-2 remains unknown, and vaccine-induced protective immunity against the infection may differ from natural immunity due to the virus's immune-evasion strategies, hence a better understanding of the natural immune response can aid in the development of effective vaccines and therapeutic strategies [13]. So, to combat such worst pandemic, increasing one's self immunity power plays a key role to boost the antibody against the microorganisms to inhibit their effect within a very few times interval.

Epidemiological research has repeatedly demonstrated that a diet rich in fruits and vegetables, as well as entire grains consumption is closely linked to a lower risk of acquiring illness [14]. And plants have been known to man since the dawn of time, and they have been used in a variety of ways throughout the times. In the ancient times Primitive humans in search for sustenance and to cope fruitfully with human miseries began to segregate plants suitable for therapeutic purpose and with defined pharmacological actions. It is estimated that, about 80 percent of population in both developing and developed countries dependent on herbal medication [15]. The plant components that play important role to provoke the immunity is known as the secondary metabolites or phytochemicals. These

secondary metabolites are active ingredients that have therapeutic properties and are therefore considered medicines or drugs [16]. They are non-nutrient bioactive plant components found in fruits, vegetables, cereals, and other plant materials that have been related to a lower risk of major chronic diseases [17]. Pharmacologically active compounds are known as phytochemicals which includes alkaloids, flavonoids, tannins, terpenoids and other volatile oils etc. Terpenoids contain antiviral, anthelmintic, antibacterial, anticancer, antimalarial, and anti-inflammatory effects, while alkaloids have antispasmodic, antimalarial, analgesic, and diuretic effects. Other phytochemicals also have antimicrobial and antiviral activities [18, 19].

For prevention and treatment of infectious diseases the use of plant extract compound is a traditional and reliable method [20]. In addition to other herbs *Elettaria cardamomum*is the most effective spice so it is also called as "queen of spices", which comes under the family *Zingiberaceae*[21]. *Elettaria cardamomum* is reported to embrace antimicrobial properties in it, andtrigger the immune system against the microorganisms [22]. As this herb is also effective against gastrointestinal disorders, high blood pressure, cardiac complication, it can be used as a remedy to current hostilities. The current study focuses on the ability of aparticular phytochemical of *Elettaria cardamomum* to defend against the symptoms caused by SARS-Co-V-2 virus by inhibiting a protein pathway which can lead to the control of the viral growth.

MATERIALS AND METHODS

Software used

The software used in this study for analysing the interaction between the phytochemical and the protein molecule is Discovery studio module which is a constituent of BIOVIA software. The software analyses the molecular interaction in between the phytochemical and the protein molecule by using the machine learning process.

List of Phytochemicals

Phytochemicals are non-nutritive chemicals present in plant having the potentials to find against certain diseaseor they have disease-preventive properties. These are the non-essential nutrients produced primarily by plants to protect them from the infective microorganisms. Nutraceutical properties that are present in the phytochemicals show a number of significant and beneficial effects on the human health condition, they also offer protection against a list of diseases and disorders like cardiovascular disease, high blood pressure, inflammation, microbial infection, parasitic and viral infections [23]. Researches have provided evidence of the presence of several phytochemicals such as phenols, terpenoids, tannins, flavonoids, etc in the herb *Elettaria cardamomum* [24].

Enzymes found in SARS-Co-V-2:

For survival of organism's energy is required which is generated from the metabolic process thatoccurs in the organism. Regulation of those metabolic processes require some enzymes to carry out the reaction. So ultimately enzymes play an important role in the lifecycle of an organism. RCSB database was used to identify the enzymes responsible for regulating the lifecycle of covid-19 causing virus. It has been found that SARS-Co-V-2 RNA-dependent RNA polymerase has a protein data base code of 6M71, which is responsible for its replication process and is the key enzyme required for the survival of the virus.

Molecular Docking

Using molecular docking methodology, the interaction between phytochemicals extracted from plant and viral proteins were studied. The phytochemical acts as a ligand molecule and establishes a strong covalent connection with the viral protein to successfully suppress the microorganism. Discovery studio module of BIOVIA software was used to detect molecular interactions and execute molecular docking to identify the interaction strength or the binding affinity between the ligand and the receptor molecule. For executing such process, the structure data file (SDF) of the phytochemicals of plant *Elettaria cardamomum* were downloaded from PUB CHEM and the PDB files of

enzymes were downloaded from RCSB. The activation of binding site of the enzyme was performed following "the receptor cavity" tab present in the receptor ligand interaction menu. The CDOCKER protocol of BIOVIA software was used to perform molecular docking under the "receptor-ligand interaction" category. The phytochemical was treated as the ligand, whereas the enzyme acted as the receptor. The "-CDOCKER ENERGY" and "-CDOCKER INTERACTION ENERGY" parameters were employed as quality indicators for molecular docking. The strong positive value of those markers indicated that the ligand and receptor had a good interaction. As a result, interactions with high values indicates key phytochemical involved in the inhibition of protein associated with the disease.

RESULTS AND DISCUSSION

CDOCKER is a simulated-annealing-based molecular dynamics (MD) algorithm. It is a grid-based molecular docking approach that is been fine-tuned for precision. Fig:1 shows the active site of the enzyme which providesan enhanced ability for attachment site of the ligand molecule to give more accurate binding strength. Molecular Dynamic techniques were used to acquire the ligand conformations. The CDOCKER energy was measured using the internal ligand strain energy and the receptor-ligand interaction energy after completion of the molecular interaction process. The energy of the nonbonded interaction between the ligand and the protein is denoted by the –CDOCKER energy. The optimal interaction conditions were determined by a) a high rate of positive value of-CDOCKER energy and b) a small difference between-CDOCKER energy and -CDOCKER interaction energy.

Table 1 reveals that the interaction of Acetic acid with RNA-dependent RNA polymerase has the highest positive value of-CDOCKER energy, which is 16.38 and owns a minimum difference of 1.39 between-C DOCKER interaction energy and-C DOCKER energy, which is followed by the interaction of Cinnam aldehyde with RNA-dependent RNA polymerase. Based on the findings, acetic acid can efficiently deactivate RNA-dependent RNA polymerase enzyme, blocking the virus's replication process, which inhibits the growth of the virus. With higher positive values, the probability of effect of the phytochemical on the viral enzyme for deactivation increases. And the negative value of the phytochemical implies a minimum effect against the viral enzyme for deactivation process.

CONCLUSION

It was noted that the plant *Elettaria cardamomum* has activities to inhibit the RNA-dependent RNA polymerase present in SARS-Co-V-2 virus. The purpose of this research was to identify the phytochemical responsible for its therapeutic properties. A molecular docking procedure was done using BIOVIA Discovery Studio module to identify the phytochemical acetic acid which has the potential to interact with a crucial enzyme RNA-dependent RNA polymerase. It was concluded from the molecular analysis that acetic acid can have a strong binding affinity or interaction with the viral enzyme followed by Cinnamaldehyde to successfully inhibit the replication process of the virus. So this study could explain that the presence of phytochemical acetic acid and Cinnamaldehyde in the plant *Elettaria cardamomum* can provide therapeutic properties against covid-19 caused by SARS-Co-V-2 virus.

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REFERENCES

1. Velavan TP, Meyer CG. The COVID-19 epidemic. Trop. Med. Int. Health 2020, 25: 278-280.
2. Spagnuolo G, Vito DD, Rengo S, Tatullo M. COVID-19 Outbreak: An Overview on Dentistry. Int. J. Environ. Res 2020.

3. Public Health Wales, Cardiff, UK. Preventing a covid-19 pandemic. BMJ 2020.
4. Yang L, Liu S, Liu J, Zhang Z, Wan X, Huang B, et al. COVID-19: immunopathogenesis and Immunotherapeutic. Signal Transduct. Target. Ther 2020.
5. Yousaf M, Zahir S, Riaz M, Hussain SM, Shah K. Statistical analysis of forecasting COVID-19 for upcoming month in Pakistan. Solitons and Fractals 2020, 138.
6. Jeffery K. History and recent advances in coronavirus discovery. Pediatr. Infect. Dis. J 2005, 24: 223-227.
7. Vittoria BL, Imbesi C, Irene G, Cali G, Bitto A. New Approaches and Repurposed Antiviral Drugs for the Treatment of the SARS-Co-V-2 Infection. Pharmaceuticals 2021, 14.
8. GISAID Global Initiative on Sharing All Influenza Data. Phylogeny of SARS-like beta coronaviruses including novel coronavirus (nCoV).
9. Chan JF, Yuan S, Kok KH, To KKW, Chu H, Yang J, Xing F, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020, 395: 514-523.
10. Zhang W, Du R, Li Bei L, Zheng XS, Yang XL, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. EmergMicrob Infect 2020, 9: 386-389.
11. Chen WL, Lan Y, Yuan XZ, Deng X, Li Y, Cai X, et al. Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. EmergMicrob Infect 2020, 9: 469-473.
12. Li T, Lu H, Zhang W. Clinical observation and management of COVID-19 Patients. Emerg. microbes & infect 2020, 9.
13. Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. Nat. Rev. Immunol 2020, 20: 615-632.
14. Liu RH. Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action. ASN2004, 134: 12.
15. Asressu KH. Antimicrobial Activity and Phytochemical Screening of Crude Extracts Of Medicinal Plants Grown In Eastern Ethiopia. Int J Pharm Bio Sci 2013, 4: 326-333.
16. Shakya AK. Medicinal plants: Future source of new drugs. Int. J. Herb. Med 2016, 4: 59-64.
17. Liu R. Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. Am. J. Clin. Nutr 2003, 78: 517-520.
18. Maurya R, Singh G, Yadav PP. Antiosteoporotic agents from Natural sources. In: Atta-ur-Rahman (Ed.) Studies in Natural Products Chemistry 2008, 35: 517-545.
19. Chopra A, Doiphode V. Ayurvedic medicine: Core concept, therapeutic principles and current relevance. Med. Clin. N. Am. 2002, 86: 75-89.
20. Moulana-Hacene F, Boufadi MY, Keddari S, Homrani A. Chemical Composition and Antimicrobial Properties of Elettaria cardamomum Extract. Pharmacogn J 2020, 12: 1058-1063.
21. Amma KPAP, Rani MP, Sasidharan I, Nisha VPN. Chemical composition, flavonoid-phenolic contents and radical scavenging activity of four major varieties of cardamom. Int J Biol Med Res 2010, 1: 3.
22. Al-Zuhair H, Ei-Sayeh B, Ameen HA, Ai-Shoora H. Pharmacological studies of cardamom oil in animals. Pharmacol Res 1996, 34: 79-82.
23. Prakash D, Gupta C, Sharma G. Importance of Phytochemicals in Nutraceuticals. J. Chin. Med. Res 2012, 1: 70-78.
24. Sharma S, Sharma J, Kaur G. Therapeutic uses of Elettaria cardamom. Int J Drug Formul Res 2011, 2: 6.

Table:1 Result of CDOCKING of the phytochemical with RNA-dependent RNA polymerase

SL. NO.	NAME OF LIGAND	VALUE OF - C DOCKER ENERGY	VALUE OF - C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Acetic acid	16.38	14.99	1.39
2	Cinnamaldehyde	-169.59	-95.24	74.35

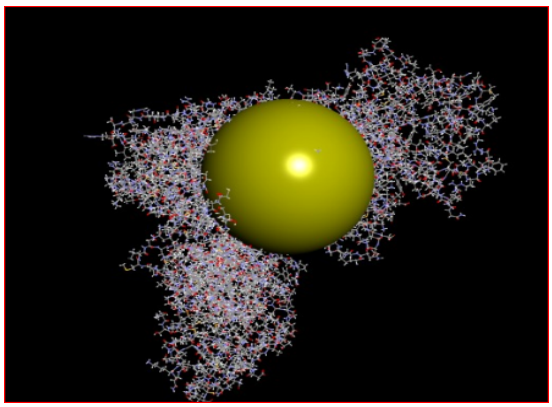

Fig:1 Active site of enzyme RNA-dependent RNA polymerase

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RESEARCH ARTICLE

Prevalence of Gram-Negative Uropathogens with Antibiogram Study in a Tertiary Care Hospital in Eastern India

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ABSTRACT

Antibiotic resistance is increasingly challenging in healthcare settings globally. Indiscriminate and overuse of antibiotics have led to multidrug resistance in various strains of Gram negative bacteria. The aim of the present study was to record the prevalence and the antibiotic resistance pattern in isolated uropathogens from catheterised patients in a tertiary care hospital. A total of 500 samples were collected from those patients admitted in the hospital, 56% (280) of the samples showed positive growth and rest 44% (220) of the samples showed no growth. In this episode of urinary tract infections, it had been seen that the women were more prone towards the infection than men. Among the bacterial isolates *Klebsiella* was found to be more prevalent followed by *Pseudomonas* and *Acinetobacter*. Antibiograms of these UTI causing bacteria were recorded in the current study which indicated moderately higher number of strains were resistant to each antibiotic used and creating the fear of precipitating intense episodes both in community and hospital settings with pathogens *Klebsiella*, *Pseudomonas* and *Acinetobacter*. Moreover, prevalence of ESBL and carbapenemase producing MDR pathogens is a matter of concern in the current era.

Keywords: Multidrug resistance, *Klebsiella, Pseudomonas,* Acinetobacter, Antibiograms, Carbapenemase.

INTRODUCTION

Human urinary tract infections (UTIs) are the most prevalent infection in humans, impacting millions of people each year. They are the second most common infection in humans, after respiratory tract infections [1]. Every year, 150-250 million cases of urinary tract infection (UTI) are reported over the world [2,3]). More than half of all women will suffer at least one UTI episode in their lives [4]. Furthermore, women are more likely (20-40%) to get recurring UTIs

(rUTIs) following an initial episode of UTI [5]. Adult women are 30 times more likely than men to get a urinary tract infection [6]. UTIs are most common in sexually active young women. UTIs are a prevalent source of infection in children and infants, and the most common bacterial infection in children under the age of two years is UTI.

The microbiota, also known as normal flora, is a large part of the human body. Each human cell contains various microorganisms, the great majority of which are not pathogens and digest complex carbohydrates, generate energy, synthesis vitamins, educate our immune system, and protect us from invading pathogens [7]. However, the microflora of a single human cell can sometimes be pathogenic to other human cells. For eradicating the infection, preventing urosepsis, and lowering the danger of renal scarring, early detection and fast and effective antimicrobial treatment are critical. *Klebsiella, Proteus, Citrobacter*, and *Pseudomonas aeruginosa* are Gram negative bacteria that cause UTIs. *Enterococcus* and *Staphylococcus saprophyticus* are Gram positive bacteria that cause UTI in young, sexually active women. *Klebsiella pneumoniae* is among the most important causes of UTI, clinically relevant pathogen that has an inclination to multidrug resistance (MDR) limiting the therapeutic options for treating the same [8]. *K. pneumonia*e is one of the top three pathogens associated with UTI as per the documentation held in 2017 by World Health Organisation's (WHO's) Global priority list of Antibiotic resistant Bacteria to guide Research, Discovery and Development of new antibiotics [9] and it is the second most frequent pathogen involved in UTI [10, 11]. Various virulence factors are used for survival and immune evasion during infection and the factors associated are specifically capsule polysaccharides, adhesins and determinants for iron accretion [12]. *Klebsiella pneumonia* is an opportunistic pathogen that infects the immunesupressed patients and tend to cause hospital acquired infections [13].

Klebsiella pneumonia is a gram-negative bacillus with a high level of antibiotic resistance. UTIs are one of the most common diseases in hospitalised patients, with *K. pneumonia* accounting for 7-10% of all UTIs [5]. *K. pneumonia* causing UTI are linked to a high rate of mortality in hospitalised patients, which rises even more in those with severe coexisting conditions including chronic renal failure, advanced liver disease, or diabetes mellitus. The appropriate antibiotic treatment of these illnesses determines the best clinical outcome, thus sensitivity testing and changing empirical antimicrobial therapy based on the results is critical.

MATERIALS AND METHODS

Collection of sample

Urine samples were collected from ICU patients in a Bhubaneswar, Odisha, tertiary care hospital. Upon admission to the ICU, most patients are catheterized. Because catheterization might cause infections, these patients' urine was collected with extreme caution using aseptic procedures. A 10ml disposable syringe was used to draw urine from the catheters. To avoid infection, the region was sterilised before the syringe was inserted.

Sample processing

The samples were collected and kept at 4°C for 24 hours before being analysed [14]. To test microbial growth, urine samples were inoculated in various culture media. The urine sample was inoculated on the Cystine-Lactose-Electrolyte Deficient (CLED) agar and blood agar medium using the streak plate method utilising a calibrated loop of 1L dipped in vertical position in the urine sample [4]. The inoculation plates were incubated for 24 hours at 37°C, then for 48 hours if the results were negative [4]. The characteristics of colonies on media for samples that grew well were evaluated and noted. The microbial isolates were tested once again. The microbial isolates were again cultured on Nutrient Agar plates to obtain a pure culture for further biochemical tests and were sub cultured periodically.

Biochemical testing for identification of microbial isolates

For biochemical testing, a single colony from pure cultures was employed. To begin, a Gram's stain was used to distinguish between Gram positive and Gram negative bacteria [4]. Gram positive bacteria were tested for catalase

and coagulase; Gram negative bacteria were tested for oxidase; and lactose fermenting bacteria were tested for Indole, Methyl Red, Voges-Proskauer, Citrate (IMViC test), Triple Sugar Iron (TSI), and Urease.

Antibiotic susceptibility testing

The conventional Kirby Bauer's disc diffusion method was used to evaluate isolates for antibiotic susceptibility [4, 15]. Standard inoculums were generated by plating 1-2 colonies on Mueller Hinton Agar (MHA) plates with a spreader after being adjusted to 0.5 McFarland. An automatic disc dispenser was used to put antimicrobial-impregnated discs onto the medium. MHA plates were then incubated for 24 hours at 37°C. The clear inhibitory zones were assessed and interpreted by clinical and laboratory standards (CLSI, 2015) after 24 hours to check each microbial isolate's resistance pattern. For isolates, the following antibiotic discs were used: Amikacin (AK) 30mcg, Cefoperazone (CPZ) 75mcg, Cefoperazone/Sulbactam (CFS) 50/50mcg, Ceftriaxone (CTR) 30mcg, Ceftazidime (CAZ) 30mcg, Co-Trimoxazole (COT) 25mcg, Clarithromycin (CLR) 30mcg, Colistin (CL) 10mcg, Gentamicin (GEN) 10mcg, Imipenam/Cilastin (IC) 10/10mcg, Levofloxacin (LE) 5mcg, Linezolid (LZ) 30mcg, Moxifloxacin (MO) 5mcg, Norfloxacin (NX) 10mcg, Ofloxacin (OF) 5mcg, Tetracycline (TE) 30mcg, Ticarcillin (TI) 75mcg, Tobramycin (TOB) 10mcg.

RESULTS AND DISCUSSION

Identification of microbial isolates

All the gram negative bacteria were subjected to oxidase test. A positive oxidase test indicated it to be a *Pseudomonas* species. Their colonies appeared to be sticky, having a fruity smell. Isolates having negative oxidase results were subjected to Methyl red test. Negative Methyl red test indicated it to be *Acinetobacter* (Figure 1). Lactose fermenting bacterial isolates appeared to be yellow in color when grown on CLED agar. The IMViC test showing a '--++' with TSI being acid with abundant gas and urease positive indicated it to be *K. pneumoniae*. Colonies appeared to be large, dome-shaped, mucoid colonies of varying degrees of stickiness.

Incidence of UTI

A total of 500 samples were collected from ICU out of which 56% (280) of the samples showed positive growth and rest 44% (220) of the samples showed no growth. Out of the 500 samples, 174 and 326 samples belonged to the females and males respectively. Incidence of UTI in females showed higher prevalence i.e. 74.14% (129 samples) to that of males being only 46.32% (151 samples). Among the bacterial isolates (Table 1), *Klebsiella* species were found to be showing higher prevalence i.e. 37.5% (105) in both males and females followed by *Pseudomonas* sp. i.e. 16.07% (45) and *Acinetobacter* i.e. 8.21% (23). Incidence of *K. pneumoniae* and was found to be higher in females i.e. 84.44% and 50.47% respectively. However, *Acinetobacter* was found infecting males i.e. 65.21% more than females.

K. pneumoniae showed no resistance to Colistin and 100% resistance to other antibacterial used in case of males, whereas in females it showed less resistance to Colistin and Imipenam/Cilastin (both 40%) followed by Tetracycline (60%), Tobramycin (60%), Linezolid (80%), Amikacin (80%), and Levofloxacin (80%) (Table 2). *Pseudomonas* sp. showed less resistance to Colistin (44.4), followed by Tetracycline (55.5%), Linezolid, Tobramycin, Imipenam/Cilastin (each 66.6%), Amikacin, Co-trimoxazole (each 77.7%) and Norfloxacin, Levofloxacin, Ceftriaxone (each 88.8%) in case of males, whereas in females it showed less resistance to Tetracycline (14.2%), followed by Imipenam/Cilastin (42.8%), Colistin, Co-trimoxazole, (each 57.1%), Cefoperazone, Cefoperazone/Sulbactum, Ceftriaxone, Ofloxacin, Levofloxacin, Norfloxacin, Gentamycin, Tobramycin, Amikacin, Linezolid (each 71.4), Moxifloxacin, Ceftazidime (each 85.7%) (Table 2).

Acinetobacter showed less resistance to Colistin, Tetracycline, Imipenam/Cilastin (each 50%), whereas in case of females, Imipenam/Cilastin, Cefoperazone, Ofloxacin, Levofloxacin, Norfloxacin, Tobramycin, Amikacin, Co-trimoxazole showed no resistance (Table 2).

The study comprised of only ICU patients being infected by UTIs. Out of 500 samples collected from these ICU patients, 220 of the samples were found to have negative growths i.e. no growth of microorganisms were found to develop in these many samples. This may have been due to the antimicrobial drugs as the ICU patients are under antibiotic cover to avoid hospital acquired infections. As per this study, the females are more affected than males as the infection and colonization of microbes other than normal flora showed higher prevalence in females than in males. The difference in UTI incidence in males and females was also statistically significant. This may be possible because the length of urethra in females is shorter as compared to males, giving the pathogens an easy access. However, UTIs in these patients can be due to long term catheterization which can be one of the possible hospital acquired infections.

Among the positive growths, the bacterial isolates found were *K. pneumoniae*, *Pseudomonas* sp., *Acinetobacter*, which are not the normal flora of urinary tract; thus indicating themselves to be pathogenic in nature. However, not more pathogens were found associated with UTIs in these patients who can be again due to antibiotic covers that the ICU patients are put under. The results showed a clear incidence of *K. pneumoniae* being predominant followed by *Pseudomonas* sp. and *Acinetobacter*. For *K. Pneumonia,* colistin was found to be the least resistant drug in case of both males and females. However there was a statistical significant difference between the treatments for *Pseudomonas* sp. and *Acinetobacter* in males and females. Colistin followed by tetracycline and Imipenam / Cilastin were the least resistant antibiotics in males whereas in females Imipenam / Cilastin, Tetracycline, Colistin, Co-trimoxazole, Cefoperazone, Tobramycin, Amikacin were the least resistant drugs. So, Colistin followed by tetracycline and Imipenam / Cilastin were the most susceptible drug for males and Imipenam/Cilastin followed by Tetracycline and Colistin are the most susceptible drugs in females.

CONCLUSION

Depending on the mechanism of infection, the organisms linked with UTIs can differ. Infections can be acquired in a variety of ways, including in the community and in hospitals. Catheterization is a common cause of urinary tract infections (UTIs) in hospitalised patients. To avoid UTIs, catheterized patients must be given particular attention. Urinary tract infections (UTIs) can cause major health problems in the kidneys, including renal failure. The emergence of multidrug resistance (MDR) bacteria has exacerbated the situation.

Conflicts of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Mobley HLT, Alteri CJ. Development of a Vaccine against *Escherichia coli* Urinary Tract Infections. Pathogens 2016; 5(1): 1. doi: 10.3390/pathogens5010001.
2. Ponnusamy P, Nagappan R. Extended spectrum beta-lactamase, biofilm-producing uropathogenic pathogens and their antibiotic susceptibility patterns from urinary tract infection-an overview. Int J Microbiol Res 2013; 4(2):101-118.
3. Asadi S, Kargar M, Solhjoo K, et al. The association of virulence determinants of uropathogenic *Escherichia coli* with antibiotic resistance Jundishapur. J Microb 2014; 7(5):1-5.
4. Mishra MP, Debata NK, Padhy RN. Surveillance of multidrug resistant uropathogenic bacteria in hospitalized patients – an Indian study. Asian Pac J Trop Biomed 2013; 3, 315-324.
5. Marques C, Menezes J, Belas A, et al. *Klebsiella pneumoniae* causing urinary tract infections in companion animals and humans: population structure, antimicrobial resistance and virulence genes. J Antimicro Chemother 2019; 74(3): 594–602.
6. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 2002; 113:5S-13S.

7. WHO. World Health Day – 7 April 2011. Antimicrobial resistance: No action today, no cure tomorrow Available at: http://www.who.int/world-health-day/2011/en/index.html (accessed 27 May 2011).
8. Podschun R, Ullmann U. *Klebsiella spp.* as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998; 11:589–603.
9. Caneiras C, Lito L, Melo-Cristino J, Duarte A. Community- and Hospital-Acquired *Klebsiella pneumoniae* Urinary Tract Infections in Portugal: Virulence and Antibiotic Resistance. Microorganisms 2019; 7(5): 138.
10. Keynan Y, Rubinstein E. The changing face of *Klebsiella pneumoniae* infections in the community. Int J Antimicrob Agents 2007; 30:385–389.
11. Lin WH, Wang MC, Tseng CC, Ko WC, Wu AB, Zheng PX, Wu JJ. Clinical and microbiological characteristics of *Klebsiella pneumoniae* isolates causing community-acquired urinary tract infections. Infection 2010; 38:459–464.
12. Hennequin C, Robin F. Correlation between antimicrobial resistance and virulence in *Klebsiella pneumoniae*. Eur J Clin Microbiol Infect Dis 2016; 35:333–341.
13. Khaertynov KS, Anokhin VA, Rizvanov AA, Davidyuk YN, Semyenova DR, Lubin SA, Skvortsova NN. Virulence Factors and Antibiotic Resistance of *Klebsiella pneumoniae* Strains Isolated From Neonates With Sepsis. Front Med 2018; 5:225.
14. Linhares I, Raposo T, Rodrigues A, Almeida A. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000–2009). BMC Infectious Diseases 2013; 13:19
15. Bauer AW, Kirby WM, Sherris JC, Turck M. "Antibiotic susceptibility testing by a standardized single disk method," American Journal of Clinical Pathology 1966; 45 (4): 493-496.

Table 1: Bacterial incidence in UTI throughout the study period

Bacteria	Total	Male	Female
K. pneumoniae	105	52	53
Pseudomonas spp.	45	7	38
Acinetobacter	23	15	8

Table 2: Antimicrobial resistance (in percentage) of bacterial isolates in male and female patients

Antimicrobial class	Antimicrobial	*K. pneumoniae*		*Pseudomonas* spp.		*Acinetobacter*	
		M	F	M	F	M	F
Beta-lactam	Imipenam/Cilastin	100	40	66.6	42.8	50	0
	Ticarcillin	100	100	100	85.7	100	100
Cephalosporin	Cefoperazone	100	100	100	71.4	100	0
	Cefoperazone/Sulbactum	100	100	100	71.4	100	100
	Ceftriaxone	100	100	88.8	71.4	100	100
	Ceftazidime	100	100	100	85.7	100	100
Fluoroquinolones	Ofloxacin	100	100	100	71.4	100	0
	Levoflxacin	100	80	88.8	71.4	100	0
	Norfloxacin	100	100	88.8	71.4	100	0
	Moxifloxacin	100	100	100	85.7	100	100
Aminoglycosides	Gentamicin	100	100	100	71.4	100	100
	Tobramycin	100	60	66.6	71.4	100	0
	Amikacin	100	80	77.7	71.4	100	0
Sulfonamide	Co-trimoxazole	100	60	77.7	57.1	100	0
Oxazolidinones	Linezolid	100	80	66.6	71.4	100	100
Macrolide	Clarithromycin	100	100	100	100	100	100
Polymyxin	Colistin	0	40	44.4	57.1	50	100
Tetracyclines	Tetracycline	100	60	55.5	14.2	50	100

Figure 1. Culture of *Acinetobater* sp. In nutrient agar medium from urine sample

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RESEARCH ARTICLE

Phytoremediation of Copper using Local Available Plant

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ABSTRACT

The present study revealed the impact of various concentrations of copper on the growth of *Capsicum annuum* L. And *Mirabilis jalapa* L. The results showed that the morphometric features such as root length, shoot length, leaf area, fresh weight and dry weight of *Capsicum annuum* L. were significantly decreased with increasing concentration of heavy metal but the growth factors were high in *Capsicum annuum* L. when it was cultivated togather with *Mirabilis jalapa* L. than the *Capsicum annuum* L. grown individually. Thus, from the above findings, it is clear that the plants, *Mirabilis jalapa*L. acted as hyperaccumulators and because of the phytoextraction capability of this hyperaccumulator, the experimental plant, *Capsicum annuum* L. (hypoaccumulator) may grow well in metal stressed environment.

Keywords : Heavy metals, copper, *Capsicum annuum* L. and*Mirabilis jalapa* L. growth.

INTRODUCTION

Heavy metal contamination of soils is a major environmental problem worldwide (CI 2011) and phytoextraction has emerged as a potential cost-effective and environmentally sustainable technique for removing toxic metals from soils (Eriksson *et al*. 1997). Phytoextraction and phytoremediation have been applied successfully for cleaning – up soils contaminated with metals from tannery sludge. Copper is the third most used metal in the world (Martínez and Motto 2000). Cu is required for the good growth of both the plants and animals. In humans, it helps in the production of hemoglobin in the blood. In plants, Cu is essential for seed production, disease resistance and pollination control. Cu is essential for life, but in high doses it can cause anemia, liver and kidney damage, and irritation of the gastrointestinal tract and intestines. Copper is often found in drinking water in copper pipes and is an anti-algae additive. The relationship between soil and water pollution and the uptake of metals by plants is determined by many chemical and physical factors of the soil and the physiological properties of plants. Soils contaminated with trace elements of metals can pose a threat both directly due to the negative impact of metals on

plant growth and yield, and indirectly due to entering the human food chain with a potential negative impact on humans. A drop in yields of just a few percent could ultimately lead to significant losses in production and income. Some food importers are now specifying the acceptable maximum contents of metals in food, which might limit the possibility for the farmers to export their contaminated crops (Bjuhr 2007), (Liu *et al*. 2013). Phytoremediation use living plants to eliminate poisons from the environment and render them innocuous. It is a financially possible and feasible ways in regards to the remediation of our current circumstance without presenting any new pollutants. Here, we summarize some of studies carried out regarding phytoremediation of copper metal.

MATERIALS AND METHODS

Seeds of *Capsicum annuum* L. was procured from local seed centres, Sivagangai. The seeds of *Mirabilis jalapa* L. was collected form the herbal garden, Raja Doraisingam Government Arts College, Sivagangai. *Mirabilis jalapa* L. was chosen as hyperaccumulator plant whereas *Capsicum annuum* L. was chosen as a co-cultivator for this study. The effect of various concentrations of copper sulphate on the morphometric characters were analyzed on the selected plants. The experimental soil for raising the cultivars was prepared by mixing red soil, black soil and sandy soil in the ratio of 1 : 1 : 1. The prepared soil was sterilized by solar sterilization method (Handiseni *et al*. 2010) for 5 days. *Capsicum annuum* L. and *Mirabilis jalapa* L. seeds were of 10 numbers were sown in all the pots for the experimental purpose. The heavy metal of copper sulphate was treated separately in the experimental plants with different concentrations viz., 2 mM, 4 mM, 6 mM, 8 mM and 10 mM (w/v) in five replicates. The aqueous solutions of copper sulphate were applied to the soil after the development of first leaves in the seedlings. Then the plants were watered with the individual concentration of metals on every alternate days. A set of plants without heavy metal treatment was maintained as control and on 35th day growth parameters were analyzed (Arts and Marks 1971), (Abdul baki and Anderson 1973).

Phytoremediation treatment - Co-cultivation of the hypoaccumulator and hyperaccumulator

Capsicum annuum L. (hypoaccumulator) and *Mirabilis jalapa* L. (hyperaccumulator) seeds were sown to-gatherly uniformly in all the pots. Appropriate amount of copper was given separately for the experimental plants with different concentration (2 mM, 4 mM, 6 mM, 8 mM and 10 mM (w/v)) in 5 replicates. Then the plants were watered with the respective concentration of metals on every alternate days and on 35th day growth parameters were analyzed.

RESULTS AND DISCUSSION

The effect of copper sulphate on the morphometric characteristics of study plants has been presented in Tables 1 and 2. The root length, shoot length, fresh weight and dry weight of *Capsicum annuum* L. was lower and reduced with the increasing concentrations of the metals but they were high in *Capsicum annuum* L. when it was cultivated to-gather with *Mirabilis jalapa* L. Heavy metals either retard the growth of the whole plant or plant parts (Shaq and Iqbal 2006), (Shanker *et al*. 2005). The plant parts typically the roots which have direct contact with the tainted soils show fast and delicate changes in their development design. Huge impacts of number of metals (Cu, Ni, Pb, Cd, Zn, Al, Hg, Cr, As, Fe) on the development of over the ground plant parts is all around archived (Wong and Bradshaw 1982). In this work, reduction in plant height might be mainly due to the reduced root growth and furthermore, ensuing lesser supplements and water transport to the above parts of the plant. Similarly It was accounted that Cr transport to the upper part of the plant can have a direct effect on cell digestion of shoot adding to the decrease in plant stature (Shanker *et al*. 2005). Reduction of leaf growth is an important visible symptom of heavy metal stress in many plants (Prasad 1997), (Fodor 2002). Plant height and total leaf area of wheat plants decreased with increasing Cu levels in the soil (Cook 1997). Reduction in biomass of the plant may be due to the poor growth of plant in stressed environment. Similar observation was reported in *Vigna trilobata* under Aluminium and Barium stress (Arundhathi *et*

al. 2016). Heavy metals uptake, by plants using phytoremediation technology, seems to be a prosperous way to remediate heavy metals- contaminated environment. It has benefits compared than the other normally utilized ordinary technologies. A few components must be considered to achieve a superior of remediation result. The most significant factor is a suitable plant which can be utilized to take-up the toxin. Indeed, even the phytoremediation strategy is by all accounts truly outstanding elective, it likewise has a few restrictions. More research should be directed to limit this restriction to apply this procedure successfully.

REFERENCES

1. CI, Copper history Future, Van Commodities Inc., (2011), http://trademetalfutures.com/copperhistory.html
2. Eriksson J, Andersson, A, Andersson, R. (1997) "The state of Swedish farmlands," Tech. Rep. 4778, Swedish Environmental Protection Agency, Stockholm, Sweden, view at: Google Scholar
3. Martínez C E , Motto, H L (2000). "Solubility of lead, zinc and copper added to mineral soils," *Environmental Pollution*, vol. 107, no. 1, pp. 153–158, View at: Publisher Site | Google Scholar
4. J. Bjuhr, (2007). Trace Metals in Soils Irrigated with Waste Water in a Periurban Area Downstream Hanoi City, Vietnam, Seminar Paper, Institutionenförmarkvetenskap, Sverigeslantbruksuniversitet (SLU), Uppsala, Sweden,
5. Liu X, Li X, ChermaineOng SM, Chu Z. (2013). Progress of Phytoremediation: Focus on New Plant and Molecular Mechanism. J Plant Biol Soil Health. 2013;1(1): 5.
6. Handiseni, M, Sibiya, J, Ogunlela, V, Koomen, I. 2010. Evaluation of non-chemical methods of soil sterilization in Paprika (*Capsicum annum* L.) Seedlings production in the small holder farming sector of Zimbabwe. *AgriculturaTropicaetSubtropica.* 43 (2): 97-108
7. Arts, H.H, Marks,P.L.(1971). A summary table of biomass and net annual primary production in forest ecosystem. (Editor,Young, HE) Life science and Agricultural experimental station. University of Marine. Orano,Maire. USA.
8. Abdul baki, A.A, Anderson, J.D. (1973). Vigourdetermination in soybean seed by multiple criteria. Crop Science, 13: 630-633.
9. Shaq M, Iqbal M Z (2006) Tolerance of Peltophorumpterocarpum D. C. Baker Ex K. Heyne. seedlings to lead and cadmium treatment. J New Seeds 7: 83 – 94.
10. Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31: 739-753.
11. Wong MH, Bradshaw AD (1982) A comparison of the toxicity of heavy metals, using root elongation of rye grass, Loliumperenne. New Phytol91: 255 – 261.
12. Prasad, M N V. 1997. In: Trace metals. Eds. Plant Ecophysiology. John Wiley & Sons, Inc. 207-249.
13. Fodor, F. (2002). Physiological responses of vascular plants to heavy metals. In: Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Eds. Prasad M.N.V. and Strzalka, K. (Kluwer Academic. Dordrecht.). pp. 149-177.
14. Cook C.M, Vardaka, E, Lanaras, T. (1997). Concentrations of Cu, growth, and chlorophyll content of field-cultivated wheat growing in naturally enriched Cu soil. *Bulletin of Environmental Contamination and Toxicology*. 58: 248-253
15. Arundhathi, A., K. Marisamy, M. Duraipandian, R. Sevugaperumal and V. Ramasubramanian.(2016). Comparison of the Metal Toxicity due toAluminium and Barium on the Growth Attributes of *Vigna trilobata* (L.) Verde. *Bioengineering and Bioscience*, 4(4): 64-69.

Table 1. Effect of copper on the morphometric characteristics of hyperaccumulator (*Mirabilis jalapa* L.) and hypoaccumulator (*Capsicum annuum L.)*

Metal concentration	Root length(cm)			Shoot length(cm)			Leaf area (cm2)		
	Copper stress on *Capsicum annuum*	After co-cultivation		Copper *Capsicum annuum* stress on	After co-cultivation		Copper stress on *Capsicum annuum*	After co-cultivation	
		Capsicum annuum	*Mirabilis jalapa*		*Capsicum annuum*	*Mirabilis jalapa*		*Capsicum annuum*	*Mirabilis jalapa*
Control	6.75±0.03	7.5±0.06	6.5±0.055	23.45±0.32	22.45±0.71	18.54±0.54	12.34±0.34	12.1±0.08	9.8±0.04
2mM	6.25±0.07	7.1±0.03	6.2±0.84	20.78±0.08	21.45±0.76	16.45±0.61	10.34±0.78	11.34±0.06	9.7±0.21
4mM	6.25±0.07	6.8±0.05	5.7±0.04	18.78±0.07	20.56±0.64	15.56±0.68	9.83±0.078	10.34±0.34	8.9±0.04
6mM	5.5±0.07	5.9±0.08	5.2±0.08	16.75±0.07	18.45±0.58	14.87±0.46	9.1±0.07	9.87±0.09	8.2±0.06
8mM	4.5±0.06	4.8±0.04	4.5±0.06	15.65±0.02	17.09±0.45	13.29±0.37	8.75±0.56	8.89±0.45	7.5±0.08
10mM	3.5±0.09	4.1±0.05	3.9±0.22	11.23±0.08	13.23±0.36	12.67±0.29	7.56±0.08	9.34±0.051	6.2±0.58

All the values are the averages of five observations. Mean ± Standard error

Table 2. Effect of copper on the morphometric characteristics of hyper accumulator (*Mirabilis jalapa*L.) and hypoaccumulator (*Capsicum annuum*L.)

Metal concentration	Fresh weight (gm)			Dry weight (gm)		
	Copper stress on *Capsicum annuum*	After Co-cultivation		Copper stress on *Capsicum annuum*	After Co-cultivation	
		Capsicum annuum	*Mirabilis jalapa*		*Capsicum annuum*	*Mirabilis jalapa*
Control	2.1±0.098	3.3±0.045	3.8±0.12	0.32±0.07	0.42±0.034	4.5±0.023
2mM	1.95±0.08	3.1±0.034	3.3±0.23	0.25±0.045	0.43±0.045	4.3±0.034
4mM	1.5±0.065	2.8±0.098	3.0±0.23	0.21±0.034	0.41±0.098	4.2±0.076
6mM	1.2±0.06	2.6±0.045	2.9±0.56	0.18±0.062	0.39±0.034	3.8±0.056
8mM	0.99±0.056	2.1±0.023	2.8±0.67	0.15±0.058	0.35±0.054	3.2±0.076
10mM	0.87±0.05	1.9±0.098	2.5±0.45	0.12±0.056	0.33±0.078	2.8±0.098

All the values are the averages of five observations. Mean ± Standard error

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RESEARCH ARTICLE

In silico Molecular Docking Studies of Oleanolic Acid from *Cichorium intybus* (Chicory) against *Yersinia pestis* Causing Plague

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ABSTRACT

Phytochemicals are bio-active chemical compounds which are found in different parts of plants. Microorganisms are pervasive which can cause different kinds of infections including Cholera. Here in this *in silico* study it has been accounted that *Cichorium intybus* (chicory) plant extract is utilized to treat Plague. It is an infectious disorder caused by *Yersinia pestis*, a gram-negative bacterium. This is a transmitted disorder, transmitted through the fleas of animals as a zoonotic bacterium. It can be transmitted from animal to human and other mammals. This *Y. pestis* is a gram-negative bacterium which can cause untreatable infection turned in to multidrug resistant. In this docking study, we discovered phytochemicals from chicory plant are effective against the protein compound of gram-negative *Y. pestis* bacteria.We generally assumed that, in silico docking, the computational devices help to discover healing therapy as a medicine against chronic cholera utilizing phytochemicals. The AutoDock Vina permits the client to upload a structure record as a pdb format for a protein and ligand and provide an outcome.

Keywords: Phytochemicals, AutoDock Vina, *Yersinia pestis, Cichorium intybus*

INTRODUCTION

Phytochemicals, a powerful group of compounds which belongs to the auxiliary/metabolites of plants includes an assorted range of synthetic/chemical substances like polyphenols, flavonoids, steroidal saponins, organosulphur compounds, and nutrients. They play a key role in development of plant, being Important for applicable physiological cycle, i.e., generation, advantageous affiliation, and collaborations with different creatures and the climate. Despite the fact that the majority of these compounds happen constitutively, their amalgamation can be upgraded under pressure conditions, in a way subject to the development conditions and on stressor [1-4]. More evidence affirms the essential role microorganisms and archaea play inside the coral holobiont, that is, the coral host and its related microbial local area. The bacterial segment comprises a local area of high variety, which seems to

change in structure because of disease occasions [5]. Plague is a boundless zoonotic illness that is brought about by *Yersinia pestis* and has devastatingly affected the human populace since the beginning. The disappearance of the infection is improbable because of the wide range of mammalian hosts and their specialist fleas. The flea/rodent life pattern of *Y. pestis*, a gram-negative obligate microorganism, opens it to totally different ecological conditions and has brought about some novel qualities working with transmission and contamination [6]**.** When these bacteria enter the blood stream directly and multiply there, it's known as septicaemic plague. People infected with the plague usually develop flu-like symptoms after two to six days of infection as well as also experience painful, swollen lymph glands, called buboes. These typically appear in groin, armpits, neck, or site of the insect bite or scratch.

Y. pestis is a Gram-negative coccobacillus that can cause three types of plague (bubonic, pneumonic, and septicaemic). The genus *Yersinia* is an individual from the family Enterobacteriaceae and comprises of 11 species, including three that are pathogenic in people: *Y. pestis, Y. pseudotuberculosis*, and *Y. enterocolitica*. While *Y. enterocolitica* and *Y. pseudotuberculosis* cause a self-restricting gastrointestinal sickness, *Y. pestis* causes an extreme, intense, and quickly advancing febrile ailment with huge death rates [3, 19]. Plants with medicinal properties have been utilized for quite a long time and various societies actually depend on native therapeutic plants to meet their essential medical service needs. All things considered, the wise information on plant-based cures in customary societies as traditionally progressed through experimentation and that the main fixes were very carefully passed starting with one generation then onto the next. Various preparations of this plant are utilized to treat different side effects and sicknesses. *Cichorium intybus* presents a little explored plant as far as phytochemistry and pharmacology [8].

For healing purposes, the utilisation of plant began before the humanity's set of experiences or mankind's history. Numerous advanced meds were begun from an organic source, biologically. Plants for the most part produce numerous metabolites which are biosynthetically derived from essential metabolites and comprise a significant source of numerous pharmaceutical drugs. Phytochemical investigation showed that the various parts *Cichorium intybus* contained sesquiterpene lactones (particularly lactucin, lactucopicrin, 8-desoxy lactucin, guaianolid glycosides, including chicoroisides B also, C, sonchuside C), caffeic corrosive subsidiaries (Oleanolic acid corrosive, chlorogenic corrosive, isochlorogenic corrosive, dicaffeoyl tartaric acid), inulin, sugars, proteins, hydroxycoumarins, flavonoids, alkaloids, steroids, terpenoids, oils, volatile compounds and mixtures, coumarins, nutrients and polyynes. It possessed hepatoprotective, gastroprotective, cardiovascular, cell reinforcement, hypolipidemic, anticancer, conceptive, antidiabetic, mitigating, pain relieving, soothing, immunological, antimicrobial, anthelmintic, hostile to protozoal, injury healing and numerous other pharmacological impacts [16]. For healing purposes, the utilisation of plant began before the humanity's set of experiences or mankind's history. Numerous advanced meds were begun from an organic source, biologically. Plants for the most part produce numerous metabolites, produce from essential metabolites biosynthetically, which comprises different pharmaceutical drugs sources. Various parts of *Cichorium intybus* contained sesquiterpene lactones (especially lactucopicrin, lacticin, 8-desoxy lacutin, guaianolid glycosides, including chicoroisides B likewise, C, sonchuside C), caffeic corrosive subsidiaries (Olcanolic acid corrosive).

Cichorium intybus L. is an individual member from the family Asteraceae. It is an as a significant restorative spice has been utilized Ayurveda, Unani and Siddha system of medication for infections of renal system and hepatobiliary system. Recent investigations have discovered a portion of the significant constituents in chicory, for example, caffeic corrosive subordinates called acid derivatives, fructo-oligosaccharides, flavonoids, inulin, and polyphe-nol1 *Cichorium intybus L.* (Compositae family) is a considered are widespread weed with antibacterial impact. Its habitants are side of the road, rail lines and waste grounds, blossoming period endures from June to October. Salts like sulphates and phosphates of sodium, magnesium furthermore, potassium just as potassium nitrate is present on the leaves of the plant. It's anything but a severe glycoside named cichorine. In customary medication, all portions of the plant uncommonly root and leaves are utilized as diuretic, diuretic, antibilious, antipyretic, blood refinement or purification and fortify of the stomach. It is utilized as an appetizer too as in the treatment of failure in hepatic

system, jaundice, discontinuous fever also, gentle conditions of constant skin in chronic state diseases. Chicorium intybus is called as Hindubar, Indyba in arabic, Zral in baluchisthan, Chicory in California, Bunk, Chicory in English, Kichora, Kikori in greek, Kasani in gujrathi, Kasni in hindi, Kasani in Persian, Gul, Hand in Punjabi, Kasni, Tsikorie, Kashini virai in tamil, Kasini vittulu in telugu, Kasani in urdu [11].

Generally, chicory was developed by the old Egyptians as a medicinal plant. The dried and simmered/roasted roots are utilized as coffee substitutes and added substances, young fresh leaves can be added to plates of mixed greens called salads and vegetable dishes, while extracts of chicory are utilized for the creation of fortifying refreshments, beverages. The plant was utilized customarily for the therapy of loose bowels (diarrhoea), to reinforce the prostate and other reproductive organs, for the therapy of pneumonic infection and cough, cancer, headache, for filtration of biliary tract, liver grievances, as spasmolytic, to alleviation of indications identified with gentle stomach related disorders (like sensation of stomach fullness, flatulence, and moderate absorption/digestion) and transitory loss of hunger. Among inside utilizes arelt was additionally utilized in sore throat, hemorrhoids, tuberculosis, stomach cramps, despairing, deafness, rashes and as diuretic for kids [18].

For the most part, chicory was created by the old Egyptians as a plant with medicinal values. The stewed/broiled roots are used as espresso (coffee) and added substances, new leaves are added to plates of blended greens called servings of mixed greens and vegetable dishes, while concentrates (extracts) of chicory are utilized for the production of sustaining rewards, refreshments or beverages. The plant was used usually for the treatment of free insides (the runs), to support the prostate and other conceptive organs, for the treatment of pneumonic disease and hack, malignancy, cerebral pain, for filtration of biliary tract, liver complaints, as spasmolytic, to lightening of signs related to delicate stomach related issues (like impression of stomach totality, fart, and moderate ingestion/absorption) and brief loss of craving. Among inside uses arelt was moreover used in irritated throat, hemorrhoids, tuberculosis, stomach cramps, despondent, deafness, rashes and as diuretic for youngsters [18].

The utilization of *Cichorium intybus* all around the world has a long tradition. Generally, chicory was developed by the antiquated Egyptians as a medicinal plant, coffee substitute, and vegetable harvest and was sometimes utilized for creature scavenge. This multipurpose plant contains high measures of proteins, carbs, and mineral components [9]. Inulin from chicory roots is viewed as a useful food ingredient as it influences physiological and biochemical cycles bringing about better wellbeing and decrease of the risks of numerous infections. [10]. The utilization of Cichorium intybus all around the world has a long tradition. Generally, chicory was developed by the antiquated Egyptians as a coffee substitute, medicinal plant and vegetable harvest and also utilized for creature scavenge. Presence of major number of proteins, mineral components and carbs are contained in this multipurpose plant [9]. The chicory roots having insulin is considered as useful food ingredients as it influences the cycles both physiological and biochemical bringing about a better wellbeing and the risk of numerous infections [10]. For the antibacterial activity against gram-negative pathogenic bacteria, the extracts of leaves and root of Cichorium were investigated [12]. The seeds of *Cichorium intybus* is utilised for the Ayurvedic and Unani systems of medicine. This important medicinal plant belongs to the Asteraceae family. Various medicinally phytoconstituents were present, which generally belong to carbohydrates, alkaloids, flavonoids, triterpenoids, tannis, saponins, fatty acids, volatile oils, and many more (Nandagopal and Ranjitha, 2007) [13].

MATERIALS AND METHODS

AutoDock Vina is a software programming tool used for virtual screening of drug discovery. The software used to check effectiveness of remedy against causative agent. We have chosen this medicinal plant which was found effective against plague and enzyme produced by causative *Y. pestis* organism. Oleanolic acid is a hydroxycinnamic acid, a phytochemical of phenylpropanoid class and found in various plants which otherwise known as cichoric acid. This is a derivative compound of caffeic acid as well as tartaric acid. This phytochemical 3D structure data collected

from PubChem Data base. PubChem is a data set of chemical compound atoms or molecules along with their associated activities against organic (biological) tests. The management of this system is kept up with by the National Centre for Biotechnology Information (NCBI), a segment of the National Library of Medicine, belongs to the United States National Institutes of Health (NIH). There will be no charges for PubChem to get its access, it can be accessed through a web UI. A large number, about a million of the structures of compound and unmistakable datasets can be unreservedly downloaded by means of FTP. PubChem contains various substance depictions and small molecules with less than 100 atoms and 1000 bonds. In excess of 80 data database vendors add to the developing PubChem *[14].* The Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB), the US server farm for the worldwide PDB archive and an establishing individual from the Worldwide Protein Data Bank organization, serves a huge number of information investors in the Americas and Oceania and makes 3D macromolecular construction information accessible at no charge and without limitations to millions RCSB.org clients all throughout the nation and globally, including >660 000 teachers, understudies and individuals from the inquisitive public utilizing PDB101.RCSB.org. [15].

RESULTS AND DISCUSSION

Through this virtual molecular study with the help of software tool AutoDock Vina, I got some good result with full fitness of ΔG Kcal/mol value with lupeol. The ΔG Kcal/mol value was about -7.5 (Table:1). This lesser value of ΔG indicated, the successful approach of phytochemical Oleanolic acid from *Cichorium intybus* (chicory) as an effective treatment against emerging infectious pathogenic organism. *Cichorium intybus* producing Oleanolic acid can be effective phytochemical for the treatment of plague.

CONCLUSION

In this current virtual molecular docking study, we found the great result interaction of the Oleanolic acid and against *Y. pestis***.** This study suggests that the Oleanolic acid bioactive compound present sufficientin*Cichorium intybus.* This phytochemical extracted from *Cichorium intybus* can be used as a primary approach to design a drug against the highly pathogenic organism. Further both in-vivo and in-vitro studies are required to extension of the drug design approach. This dry lab investigation proven much cost effective and less time-consuming procedure to study about the different treatments against various untreatable diseases.

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REFERENCES

1. MahajanSand TutejaN, "Cold, salinity and drought stresses: an overview," Archives of Biochemistry and Biophysics, vol. 444, no. 2, pp. 139–158, 2005.
2. ForniC, Duca D, and GlickBR, "Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria," Plant and Soil, vol. 410, no. 1-2, pp. 335–356, 2017.
3. Ramakrishna Aand RavishankarGA, "Influence of abiotic stress signals on secondary metabolites in plants," Plant Signaling and Behavior. 2011; 6(11), 1720–1731, 2011.
4. ForniC, Facchiano F,Bartoli M, Pieretti S, FacchianoA, D'Arcangelo D, Norelli S,Valle G, Nisini, Beninati S, Tabolacci C, and Jadeja RN. "Beneficial Role of Phytochemicals on Oxidative Stress and Age-Related Diseases". BioMed Research International. 2019;1-16.

5. Sunagawa S,DeSantis TZ, Piceno YM, Brodie EL,DeSalvo MK, VoolstraCR, WeilE, Andersen GL & Medina M. "Bacterial diversity and White Plague Disease-associated community changes in the Caribbean coral Montastraea faveolate". The ISME Journal. 2009; 3: 512–521.
6. PerryRD, FetherstonJD. "Yersinia pestis--etiologic agent of plague". Clin Microbiol Rev. 1997; 10(1):35-66.
7. Pechous RD, Sivaraman V, Stasulli NM, and Goldman WE. "Pneumonic Plague: The Darker Side of Yersinia pestis". Infectious Diseases. 2016;24(3):190-197.
8. Street RA, Sidana J, Gerhard Prinsloo. "Cichorium intybus: Traditional Uses, Phytochemistry, Pharmacology, and Toxicology". Hindawi Publishing Corporation. 2013;1-13.
9. Wang Q and CuiJ, "Perspectives and utilization technologies of chicory (Cichorium intybus L.): a review," African Journal of Biotechnology, vol. 10, no. 11, pp. 1966–1977, 2011.
10. Kaur Nand GuptaAK, "Applications of inulin and oligofructose in health and nutrition," Journal of Biosciences. 2002; 27(7): 703–714.
11. Roohi Zaman and Sadiya Noorul Basar. "A Review Article of Beekhe Kasni (Cichorium intybus) its Traditional uses and Pharmacological Actions". Research Journal of Pharmaceutical Sciences. 2013; 2(8), 1-4.
12. Verma R, Rawat A, Ganie SA, Agnihotri RK, Sharma R, Mahajan S, Gupta A. "In vitro Antibacterial Activity of Cichorium intybus against some Pathogenic Bacteria". British Journal of Pharmaceutical Research. 2013; 3(4): 767-775.
13. shaikh T,Rukhsana A,Sasikumar S. "Antimicrobial screening of Cichorium intybus seed extracts". Arabian Journal of Chemistry. 2016; 9:1569-1573.
14. "PubChem Source Information". The PubChem Project. USA: National Center for Biotechnology Information.
15. BurleySK , BhikadiyaC, Chunxiao Bi, Bittrich S, Chen L, Crichlow GV, Christie CH, DalenbergK, Costanzo LD, Duarte JM, Dutta S, Feng Z, Ganesan S, Goodsell DS,Ghosh S, Green RK, Guranović V, Guzenko D, Hudson BP, Lawson CL, Yuhe Liang, Lowe R, Namkoong H, Peisach E, Persikova I, Randle C, Rose A, Rose Y, Sali A, Segura J, Sekharan M, Shao C, Tao YP, Voigt M, Westbrook JD, YoungJY, Zardecki C, ZhuravlevaM. "RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences". Nucleic Acids Research. 2021;41: D437–D451.
16. Al-Snafi AE. "Medical importance of Cichorium intybus – A review". IOSR Journal of Pharmacy. 2016; 4(3): 41-56.
17. Bais HB and Ravishankar GA. "Cichorium intybus L. – Cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects". J Sci Food Agric. 2001; 81:467-484.
18. Judzentiene A, Būdiene J. "Volatile constituents from aerial parts and roots of Cichorium intybus L. (chicory) grown in Lithuania". chemija. 2008; 19 (2): 25–28.
19. Roger D. Pechous, Vijay Sivaraman, Nikolas M. Stasulli, William E. Goldman. "Pneumonic Plague: The Darker Side of Yersinia pestis", Trends in Microbiology, 2016; 24(3):190-197.

Table:1 . Affinity value with dist from best mode

mode	Affinity (kcal/mol)	dist from best mode	
		rmsd l.b	rmsd u.b.
1	-7.5	0.000	0.000
2	-6.6	28.244	31.257
3	-6.5	20.082	24.640
4	-6.4	20.617	23.011
5	-6.4	21.311	25.047
6	-6.4	22.167	25.638
7	-6.4	1.856	8.442
8	-6.4	21.502	23.868
9	-6.3	23.924	26.955

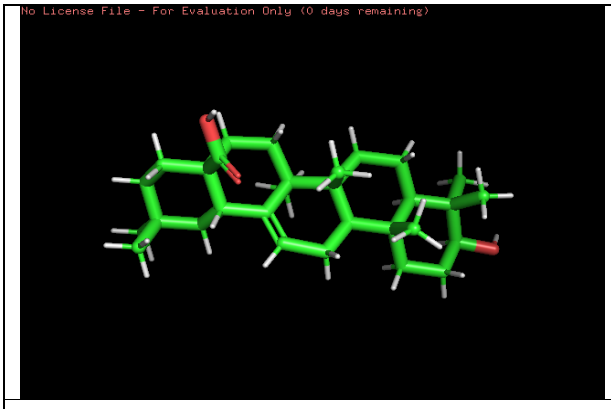

Figure 1- Ligand: Oleanolic Acid

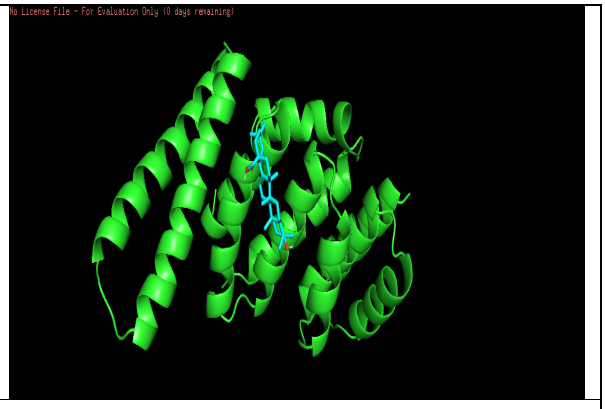

Figure 2- Active site of PDB ID-1xkp (Type III Secretion system from *Yersinia pestis*) with *Cichorium intybus*

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RESEARCH ARTICLE

Properties of (1,2)*-$\dot{\Upsilon}$-Closed Sets in Bitopological Spaces

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ABSTRACT

In this paper, we introduce a new class of sets namely(1,2)*-$\dot{\Upsilon}$-closed sets in bitopological spaces and we study some of its basic properties.
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Keywords and Phrases: (1,2)*-gs-kernel, (1,2)*-gs-closed set, (1,2)*-$\dot{\Upsilon}$-closed set.

INTRODUCTION

Kelly [6] introduced the concepts of bitopological spaces.Recently, Bhattacharya and Lahiri [5], Arya and Nour [4], Rajamani and Viswanathan [9]introduced sg-closed sets, gs-closed sets, ω-closed sets and αgs-closed sets respectively. In this paper, we introduce a new class of sets namely (1,2)*-$\dot{\Upsilon}$-closed sets in bitopological spaces and we study some of its basic properties.

PRELIMINARIES

Throughout this paper, (X, τ_1, τ_2) (briefly, X) will denote bitopological space (briefly, BTPS).

Definition 2.1 Let H be a subset of X. Then H is said to be $\tau_{1,2}$-open [12] if H = P∪Q where P∈ τ_1 and Q∈ τ_2.
The complement of $\tau_{1,2}$-open set is called$\tau_{1,2}$-closed.
Notice that$\tau_{1,2}$-open sets need not necessarily form a topology.

Definition 2.2 [12] Let H be a subset of a bitopological space X. Then

(i) the $\tau_{1,2}$-closure of H, denoted by $\tau_{1,2}$-cl(H), is defined as ∩{F : H ⊆ F and F is $\tau_{1,2}$-closed}.

(ii)the $\tau_{1,2}$-interior of H, denoted by $\tau_{1,2}$-int(H), is defined as ∪{F : F ⊆ H and F is $\tau_{1,2}$-open}.

Definition 2.3 A subset H of a BTPS X is called:
(i) (1,2)*-semi-open set [11] if H ⊂$\tau_{1,2}$-cl($\tau_{1,2}$-int(H));
(ii) (1,2)*-preopen set [11] if H ⊂$\tau_{1,2}$-int($\tau_{1,2}$-cl(H));
(iii) regular (1,2)*-open set [11] if H = $\tau_{1,2}$-int($\tau_{1,2}$-cl(H)).
The complements of the above-mentioned open sets are called their respective closed sets.

Definition 2.4 A subset H of a BTPS X is called
(i) (1,2)*-generalized closed (briefly, (1,2)*-g-cld) set [16] if $\tau_{1,2}$-cl(H) ⊆ U whenever H ⊆ U and U is $\tau_{1,2}$-open in X.
(ii) (1,2)*-semi-generalized closed (briefly, (1,2)*-sg-cld) set [11] if (1,2)*-scl(H) ⊆ U whenever H ⊆ U and U is (1,2)*-semi-open in X.
(iii) (1,2)*-generalized semi-closed (briefly, (1,2)*-gs-cld) set [11] if (1,2)*-scl(H) ⊆ U whenever H ⊆ U and U is $\tau_{1,2}$-open in X.
The complements of the above-mentioned closed sets are called their respective open sets.

Definition 2.3 [16] Subset H of a BTPS X is said to be (1,2)*-locally closed (briefly, (1,2)*-locall cld) if H = U ∩ F, where U is $\tau_{1,2}$-open and F is $\tau_{1,2}$-closed in X.

Definition 2.4 [17] A subset H of X is called a (1,2)*-$\dot{\Upsilon}$-closed (briefly, (1,2)*-$\dot{\Upsilon}$-cld) set if $\tau_{1,2}$-cl(H) ⊆ U whenever H⊆ U and U is (1,2)*-gs-open in X. The complement of the (1,2)*-$\dot{\Upsilon}$-closed (briefly, (1,2)*-$\dot{\Upsilon}$-cld) set is (1,2)*-$\dot{\Upsilon}$-open set.

PROPERTIES OF(1,2)*-$\dot{\Upsilon}$-CLOSED SETS

In this paper, we have proved that an arbitrary intersection of (1,2)*-$\dot{\Upsilon}$-cld sets is (1,2)*-$\dot{\Upsilon}$-cld. Moreover, we discuss some basic properties of (1,2)*-$\dot{\Upsilon}$-cld sets.

Definition 3.1: The intersection of all (1,2)*-gs-open subsets of X containing H is called the (1,2)*-gs-kernel of H and denoted by (1,2)*-gs-ker(H).

Lemma 3.2 : A subset H of X is (1,2)*-$\dot{\Upsilon}$-cld if and only if $\tau_{1,2}$-cl(H) ⊆(1,2)*-gs-ker(H).

Proof: Suppose that H is(1,2)*-$\dot{\Upsilon}$-cld. Then $\tau_{1,2}$-cl(H) ⊆ U whenever H⊆ U and U is (1,2)*-gs-open. Let x ∈$\tau_{1,2}$-cl(H). If x ∉(1,2)*-gs-ker(H), then there is a (1,2)*-gs-open set U containing H such that x ∉ U. Since U is a (1,2)*-gs-open set containing H, we have x ∉$\tau_{1,2}$-cl(A) and this is a contradiction. Conversely, let $\tau_{1,2}$-cl(H) ⊆(1,2)*-gs-ker(H). If U is any (1,2)*-gs-open set containing H, then $\tau_{1,2}$-cl(H) ⊆(1,2)*-gs-ker(H) ⊆ U. Therefore, H is (1,2)*-$\dot{\Upsilon}$-cld.

Proposition 3.3: For any subset H ofX, $X_2 \cap \tau_{1,2}$-cl(H) $\subseteq$(1,2)*-gs-ker(H), where X_2 = {x $\in$ X : {x} is (1,2)*-preopen}.

Proof: Let x $\in$ $X_2 \cap \tau_{1,2}$-cl(H) and suppose that x $\notin$(1,2)*-gs-ker(H). Then there is a (1,2)*-gs-open set U containing H such that x $\notin$ U. If F = X – U, then F is (1,2)*-gs-cld. Since $\tau_{1,2}$-cl({x}) $\subseteq \tau_{1,2}$-cl(H), we have $\tau_{1,2}$-int($\tau_{1,2}$-cl({x})) $\subseteq$H$\cup \tau_{1,2}$-int($\tau_{1,2}$-cl(H)). Again since x $\in$ X_2, we have x $\notin$ X_1 and so $\tau_{1,2}$-int($\tau_{1,2}$-cl({x})) = ϕ. Therefore, there has to be some y $\in$H$\cap \tau_{1,2}$-int($\tau_{1,2}$-cl({x})) and hence y $\in$ F $\cap$H, a contradiction.

Recall that a bitopological space X and H is subset of X is called (1,2)*-nowhere dense if $\tau_{1,2}$-int($\tau_{1,2}$-cl({H})) = ϕ.

Theorem 3.4: A subset H of X is (1,2)*-$\dot{\Upsilon}$-cld if and only if $X_1 \cap \tau_{1,2}$-cl(H) $\subseteq$H, where X_1 = {x $\in$ X : {x} is (1,2)*-nowhere dense}.

Proof: Suppose that H is (1,2)*-$\dot{\Upsilon}$-cld. Let x $\in$ $X_1 \cap \tau_{1,2}$-cl(H). Then x $\in$ X_1 and x $\in \tau_{1,2}$-cl(H). Since x $\in$ X_1, $\tau_{1,2}$-int($\tau_{1,2}$-cl({x})) = ϕ. Therefore, {x} is (1,2)*-semi-closed, since $\tau_{1,2}$-int($\tau_{1,2}$-cl({x})) $\subseteq$ {x}. Since every (1,2)*-semi-closed set is (1,2)*-gs-cld, Since {x} is (1,2)*-gs-closed. If x $\notin$H and if U = X \ {x}, then U is a (1,2)*-gs-open set containing H and so $\tau_{1,2}$-cl(H) $\subseteq$ U, a contradiction.

Conversely, suppose that $X_1 \cap \tau_{1,2}$-cl(H) $\subseteq$H. Then $X_1 \cap \tau_{1,2}$-cl(H) $\subseteq$(1,2)*-gs-ker(H), since H$\subseteq$(1,2)*-gs-ker(H). Now $\tau_{1,2}$-cl(H) = X$\cap \tau_{1,2}$-cl(H) = ($X_1 \cup X_2$) $\cap \tau_{1,2}$-cl(H) = ($X_1 \cap \tau_{1,2}$-cl(H)) $\cup$ ($X_2 \cap \tau_{1,2}$-cl(H)) $\subseteq$(1,2)*-gs-ker(H), since $X_1 \cap \tau_{1,2}$-cl(H) $\subseteq$(1,2)*-gs-ker(H). Thus, H is (1,2)*-$\dot{\Upsilon}$-cld.

Theorem 3.5: An arbitrary intersection of (1,2)*-$\dot{\Upsilon}$-cld sets is (1,2)*-$\dot{\Upsilon}$-cld.

Proof: Let F = {H_i : i$\in \wedge$} be a family of (1,2)*-$\dot{\Upsilon}$-cld sets and let H = $\cap_{i \in \wedge} H_i$. Since H$\subseteq H_i$for each i, $X_1 \cap \tau_{1,2}$-cl(H) $\subseteq$ $X_1 \cap \tau_{1,2}$-cl(H_i) for each i.Using Theorem 3.4, for each (1,2)*-$\dot{\Upsilon}$-cld set H_i, we have $X_1 \cap \tau_{1,2}$-cl(H_i) $\subseteq H_i$. Thus, $X_1 \cap \tau_{1,2}$-cl(H) $\subseteq$ $X_1 \cap \tau_{1,2}$-cl(H_i) $\subseteq H_i$ for each i $\in \wedge$. That is, $X_1 \cap \tau_{1,2}$-cl(H) $\subseteq$H and so H is (1,2)*-$\dot{\Upsilon}$-cld, by Theorem 3.4.

Corollary 3.6: If H is a (1,2)*-$\dot{\Upsilon}$-cld set and F is a $\tau_{1,2}$-closed set, then H$\cap$ F is a (1,2)*-$\dot{\Upsilon}$-cld set.

Proof: Since F is closed, it is (1,2)*-$\dot{\Upsilon}$-cld. Therefore by Theorem 3.5, H$\cap$ F is also a (1,2)*-$\dot{\Upsilon}$-cld set.

Proposition 3.7: If A and B are (1,2)*-$\dot{\Upsilon}$-cld setsin X, then A $\cup$ B is (1,2)*-$\dot{\Upsilon}$-cld in X.

Proof: If A $\cup$ B $\subseteq$ G and G is (1,2)*-gs-open, then A $\subseteq$ G and B $\subseteq$ G. Since A and B are (1,2)*-$\dot{\Upsilon}$-cld, G $\supseteq \tau_{1,2}$-cl(A) and G $\supseteq \tau_{1,2}$-cl(B) and hence G $\supseteq \tau_{1,2}$-cl(A)$\cup \tau_{1,2}$-cl(B) = $\tau_{1,2}$-cl(A$\cup$B). Thus A$\cup$B is (1,2)*-$\dot{\Upsilon}$-cld set in X.

Proposition 3.8: If a set His(1,2)*-$\dot{\Upsilon}$-cld in X, then $\tau_{1,2}$-cl(H) –H contains no nonempty $\tau_{1,2}$-closed set in X.

Proof: Suppose that H is (1,2)*-$\dot{\Upsilon}$-cld. Let F be a $\tau_{1,2}$-closed subset of $\tau_{1,2}$-cl(H) –H. Then H$\subseteq$ F^c. But H is (1,2)*-$\dot{\Upsilon}$-cld,therefore $\tau_{1,2}$-cl(H) $\subseteq$ F^c. Consequently, F $\subseteq$ ($\tau_{1,2}$-cl(H))^c. We already have F $\subseteq \tau_{1,2}$-cl(H). Thus F $\subseteq \tau_{1,2}$-cl(H) $\cap$ ($\tau_{1,2}$-cl(H))^c and F is empty.
The converse of Proposition 3.8 need not be true as seen from the following example.

Example 3.9: Let X = {a, b, c}, τ_1 = {ϕ, X} and τ_2 = {ϕ, X, {a}}. Then the sets in {ϕ, {a}, X}are called $\tau_{1,2}$-openand the sets in {ϕ, X,{b, c}} are called $\tau_{1,2}$-closed. Then (1,2)*-$\dot{\Upsilon}$C(X) = {ϕ, {b, c}, X}. If A = {b}, then $\tau_{1,2}$-cl(A) – A = {c} does not contain any nonempty $\tau_{1,2}$-closed set. But A is not (1,2)*-$\dot{\Upsilon}$-cld in X.

Theorem 3.10: A set H is (1,2)*-$\dot{\Upsilon}$-cld if and only if $\tau_{1,2}$-cl(H) –H contains no nonempty (1,2)*-gs-cld set.

Proof: Necessity. Suppose that H is (1,2)*-Ŷ-cld. Let S be a (1,2)*-gs-cld subset of $\tau_{1,2}$-cl(H) −H. Then H⊆ S^c. Since H is (1,2)*-Ŷ-cld, we have $\tau_{1,2}$-cl(H) ⊆ S^c. Consequently, S ⊆ ($\tau_{1,2}$-cl(H))^c. Hence, S ⊆$\tau_{1,2}$-cl(H) ∩ ($\tau_{1,2}$-cl(H))^c = ϕ. Therefore S is empty. Sufficiency. Suppose that $\tau_{1,2}$-cl(H) −H contains no nonempty (1,2)*-gs-cld set. Let H⊆ G and G be both $\tau_{1,2}$-closed and (1,2)*-sg-open. If $\tau_{1,2}$-cl(H) ⊄ G, then $\tau_{1,2}$-cl(H) ∩G^c≠ϕ. Since $\tau_{1,2}$-cl(H) is a $\tau_{1,2}$-closed set and G^c is both $\tau_{1,2}$-open and(1,2)*-sg-cld set, $\tau_{1,2}$-cl(H) ∩G^c is a nonempty (1,2)*-gs-cld subset of $\tau_{1,2}$-cl(H) −H (from Corollary 3.6). This is a contradiction. Therefore, $\tau_{1,2}$-cl(H) ⊆ G and hence H is (1,2)*-Ŷ-cld.

Proposition 3.11: If A is (1,2)*-Ŷ-cld in X and A ⊆ B ⊆$\tau_{1,2}$-cl(A), then B is (1,2)*-Ŷ-cld in X.

Proof: Since B ⊆$\tau_{1,2}$-cl(A), we have $\tau_{1,2}$-cl(B) ⊆$\tau_{1,2}$-cl(A). Then, $\tau_{1,2}$-cl(B) − B ⊆$\tau_{1,2}$-cl(A) − A. Since $\tau_{1,2}$-cl(A) − A has no nonempty (1,2)*-gs-cld subsets, neither does $\tau_{1,2}$-cl(B) − B. By Theorem 3.10, B is (1,2)*-Ŷ-cld.

Proposition 3.12: Let H⊆ Y ⊆ X and suppose that H is (1,2)*-Ŷ-cld in X. Then A is (1,2)*-Ŷ-cld relative to Y.

Proof: Let H⊆ Y ∩ G, where G is (1,2)*-gs-open in X. Then H⊆ G and hence $\tau_{1,2}$-cl(H) ⊆ G. This implies that Y ∩$\tau_{1,2}$-cl(H) ⊆ Y ∩ G. Thus H is (1,2)*-Ŷ-cld relative to Y.

Proposition 3.13: If H is a (1,2)*-gs-open and (1,2)*-Ŷ-cld in X, then H is closed in X.

Proof: Since H is (1,2)*-gs-open and (1,2)*-Ŷ-cld, $\tau_{1,2}$-cl(H) ⊆H and hence H is $\tau_{1,2}$-closed in X.

Recall that a bitopological space X is called (1,2)*-extremally disconnected if $\tau_{1,2}$-cl(U) is $\tau_{1,2}$-open for each U ∈$\tau_{1,2}$.

Theorem 3.14: Let Xbe (1,2)*-extremally disconnected and His (1,2)*-semi-open subset of X. Then H is (1,2)*-Ŷ-cld if and only if it is (1,2)*-gs-cld.

Proof: It follows from the fact that if Xis(1,2)*-extremally disconnected and H is a (1,2)*-semi-open subset of X, then (1,2)*-scl(H) = $\tau_{1,2}$-cl(H).

Theorem 3.15 : Let H be a (1,2)*-locally cld set of X. Then H is $\tau_{1,2}$-closed if and only if H is (1,2)*-Ŷ-cld.

Proof: (i) ⇒ (ii). It is fact that every $\tau_{1,2}$-closed set is (1,2)*-Ŷ-cld.

(ii) ⇒ (i). We have H∪ (X −$\tau_{1,2}$-cl(H)) is $\tau_{1,2}$-open in X, since H is (1,2)*-locally cld. Now H∪ (X −$\tau_{1,2}$-cl(H)) is (1,2)*-gs-open set of X such that H⊆H∪ (X −$\tau_{1,2}$-cl(H)). Since H is (1,2)*-Ŷ-cld, then $\tau_{1,2}$-cl(H) ⊆H∪ (X −$\tau_{1,2}$-cl(H)). Thus, we have $\tau_{1,2}$-cl(H)⊆H and hence H is a $\tau_{1,2}$-closed.

Proposition 3.16: For each x ∈ X, either {x} is (1,2)*-gs-cld or $\{x\}^c$ is (1,2)*-Ŷ-cld in X.

Proof: Suppose that {x} is not (1,2)*-gs-cld in X. Then $\{x\}^c$ is not (1,2)*-gs-open and the only (1,2)*-gs-open set containing $\{x\}^c$ is the space X itself. Therefore $\tau_{1,2}$-cl($\{x\}^c$) ⊆ X and so $\{x\}^c$ is (1,2)*-Ŷ-cld in X.

Theorem 3.17: Let H be a (1,2)*-Ŷ-cld set of a bitopological space X. Then,

(i) (1,2)*-sint(H) is (1,2)*-Ŷ-cld.
(ii) If H is regular(1,2)*-open, then (1,2)*-pint(H) and (1,2)*-scl(H) are also (1,2)*-Ŷ-cld sets.
(iii)If H is regular (1,2)*-closed, then (1,2)*-pcl(H) is also (1,2)*-Ŷ-cld.

Proof:

i. Since $\tau_{1,2}$-cl($\tau_{1,2}$-int(H)) is a $\tau_{1,2}$-closed set in X, by Corollary 3.6, (1,2)*-sint(H) = H$\cap\tau_{1,2}$-cl($\tau_{1,2}$-int(H)) is (1,2)*-$\dot{\Upsilon}$-cld in X.

ii. Since H is regular (1,2)*-open in X, H = $\tau_{1,2}$-int($\tau_{1,2}$-cl(H)). Then (1,2)*-scl(H) = H$\cup\tau_{1,2}$-int($\tau_{1,2}$-cl(H)) = H. Thus, (1,2)*-scl(H) is (1,2)*-$\dot{\Upsilon}$-cld in X. Since (1,2)*-pint(H) = H$\cap\tau_{1,2}$-int($\tau_{1,2}$-cl(H)) = H, (1,2)*-pint(H) is (1,2)*-$\dot{\Upsilon}$-cld.

iii. Since H is regular (1,2)*-closed in X, H = $\tau_{1,2}$-cl($\tau_{1,2}$-int(H)). Then (1,2)*-pcl(H) = H$\cup\tau_{1,2}$-cl($\tau_{1,2}$-int(H)) = H. Thus, (1,2)*-pcl(H) is (1,2)*-$\dot{\Upsilon}$-cld in X.

The converses of the statements in the Theorem 3.17 are not true as we see in the following examples.

Example 3.18

Let X = {a, b, c}, τ_1 = {ϕ, X, {c}} and τ_2 = {ϕ, X, {b, c}}. Then the sets in {ϕ, {c}, {b, c}, X}are called $\tau_{1,2}$-openand the sets in {ϕ, X, {a}, {a, b}} are called $\tau_{1,2}$-closed. Then (1,2)*-$\dot{\Upsilon}$C(X) = {ϕ, {a}, {a, b}, X}.Then the setH = {b} is not a (1,2)*-$\dot{\Upsilon}$-cld set. However (1,2)*-sint(H) = ϕ is a (1,2)*-$\dot{\Upsilon}$-cld.

Example 3.19

Let X = {a, b, c}, τ_1 = {ϕ, X, {a}} and τ_2 = {ϕ, X, {a, b}}. Then the sets in {ϕ, {a}, {a, b}, X}are called $\tau_{1,2}$-openand the sets in {ϕ, X, {c}, {b, c}} are called $\tau_{1,2}$-closed. Then (1,2)*-$\dot{\Upsilon}$C(X) = {ϕ, {c}, {b, c}, X}. Then the set H = {c} is not regular (1,2)*-open. However H is (1,2)*-$\dot{\Upsilon}$-cld and (1,2)*-scl(H) = {c} is a (1,2)*-$\dot{\Upsilon}$-cld and (1,2)*-pint(H) = ϕ is also (1,2)*-$\dot{\Upsilon}$-cld.

Example 3.20

In Example 3.19, the set H = {c} is not regular(1,2)*-closed. However H is a (1,2)*-$\dot{\Upsilon}$-cld and (1,2)*-pcl(H) = {c} is (1,2)*-$\dot{\Upsilon}$-cld.

REFERENCES

1. Abd El-Monsef, M. E., El-Deeb, S. N. and Mahmoud, R. A.: β-open sets and β-continuous mapping, Bull. Fac. Sci. Assiut Univ, 12(1983), 77-90.
2. Andrijevic, D.: Semi-preopen sets, Mat. Vesnik, 38(1986), 24-32.
3. Arya, S. P. and Nour, T.: Characterization of s-normal spaces, Indian J.Pure. Appl. Math., 21(8)(1990), 717-719.
4. Bhattacharya, P. and Lahiri, B. K.: Semi-generalized closed sets in topology, Indian J. Math., 29(3)(1987), 375-382.
5. Duszynski, Z., Jeyaraman, M., Joseph Israel, M. and Ravi, O.: A new generalization of closed sets in bitopology, South Asian Journal of Mathematics, 4(5)(2014), 215-224.
6. Kelly, J.C. : Bitopological spaces, Proc.London Math.Soc., 3(13)(1963), 71-89.
7. Levine, N.: Generalized closed sets in topology, Rend. Circ. Math. Palermo, 19(2)(1970), 89-96.
8. Lellis Thivagar, M., Ravi, O. and Abd El-Monsef, M. E.: Remarks on bitopological (1,2)*-quotient mappings, J. Egypt Math. Soc., 16(1) (2008), 17-25.
9. Rajamani, M., and Viswanathan, K.: On αgs-closed sets in topological spaces, Acta Ciencia Indica, XXXM (3)(2004), 21-25.
10. Ravi, O., Thivagar, M. L. and Hatir, E.: Decomposition of (1,2)*-continuity and (1,2)*-α -continuity, Miskolc Mathematical Notes., 10(2) (2009), 163-171.
11. Ravi, O. and Lellis Thivagar, M.: A bitopological (1,2)*-semi-generalized continuous maps, Bull. Malays. Math. Sci. Soc., (2), 29(1) (2006), 79-88.
12. Ravi, O. and Lellis Thivagar, M.: On stronger forms of (1,2)*-quotient mappings in bitopological spaces, Internat. J. Math. Game Theory and Algebra., 14(6) (2004), 481-492.
13. Ravi, O. and Thivagar, M. L.: Remarks on λ-irresolute functions via (1,2)*-sets, Advances in App. Math. Analysis, 5(1) (2010), 1-15.

14. Ravi, O., Pious Missier, S. and Salai Parkunan, T.: On bitopological (1,2)*-generalized homeomorphisms, Int J. Contemp. Math. Sciences., 5(11) (2010), 543-557.
15. Ravi, O., Pandi, A., Pious Missier, S. and Salai Parkunan, T.: Remarks on bitopological (1,2)*-rω-Homeomorphisms, International Journal of Mathematical Archive, 2(4) (2011), 465-475.
16. Ravi, O., Thivagar, M. L. and Jinjinli.: Remarks on extensions of (1,2)*-g-closed maps,Archimedes J. Math., 1(2) (2011), 177-187.
17. Revathi, P., Shanmuga Vadivu, C., Kavitha, K., Banumathi, K. and Pandi, A.: (1,2)*-Ῠ-Closed Sets in Bitopological Spaces, International Journal of Scientific Research and Engineering Development, 4(3) (2021), 328-333.

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RESEARCH ARTICLE

Shadow Link Processor: A Hybrid Model Web Intrusion Detection System

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ABSTRACT

In the recent trend of network communications, each user accessing unidentified and unauthorized web sites. Since most of victim doesn't know the path where they are moving through to get their responses, many of them are indulging with non-secured access with their system and sensitive data which they are transmitting to communicate. Those anonymous connectivity from various places and countries have been held through one black digital market infrastructure which is known as "Dark Web". As the name specified, in this "Dark Web" no one can identify the communicator's details such as source and destination IP addresses, Ports, Agent systems etc. Since those indexing matters are not available to access the personal information, nobody can expose the identity of source and destination users. This leads to exploit the security function of the systems and safety of the data which are being communicated. In this paper, the following concepts are going to be discussed. 1) Dark Web 2) Tor Architecture 3) Intrusion Attacks and 4) finally a hybrid model web intrusion detection system using shadow link processing algorithm. This hybrid model works on two different functions as dataset feature selection and web crawler's log event analysis.

Keywords: Dark Web, Intrusion Attacks, Intrusion Detection System, Network Communication Model, Onion, Shadow Link Processor, The Onion Router (ToR).

INTRODUCTION

With the growing usage of networking technology, many hackers are trying to evade the user data from their personal data transmission. In the earlier stages, user hacks the data via spam information, spam image and hidden submission links. Later many researchers hardly worked to bring perfect solutions on their spoofing techniques. Recently hackers shifted their ideas to configuring the proxy servers. All those proxy servers and malicious links, targets have been deployed in a non-secured environment and this place is called as "Dark Web". Network model contains two important generic architecture to carry the data transmission such as 1) ISO/OSI network model and 2) TCP/IP protocol model. In the aforementioned model, layering structure was imposed to perform variety of operations based on each layer methods. Those layers are functioning on gathering user data as raw bits to constructing them back into the same format to present at the receiver [1]. Through these layers only all data transmissions are happened. Hackers uses those layers to spoofing the communication systems and extracting the data from the users. Intrusion detection became an emerging area of research. Intrusion Detection System (IDS) attempts to identify and notify the activities of users as normal (or) anomaly. IDS is a nonlinear, complicated problem and deals with network traffic data. Many IDS methods have been proposed and produce different levels of accuracy. This is why development of effective and robust Intrusion detection system is necessary. Many classifiers are used to identify the parameters of an intrusion attacks [2].

Dark Web -Tor Browser

In the year of 2002, US navy research collaborated with other research organizations such as Electronic Frontier foundation, Knight Community body foundation, Swedish International Development corporation agency and etc., come front to develop Tor project. The Onion Routing (Tor) project widely used to maintain privacies of the corporate users rather than government agencies. Onion routing is accomplished using communication protocol stack application layer encryption nested as the onion layer. Tor encrypts data multiple times, including the destination IP address of the next hop, and sends it over a virtual channel consisting of a sequentially randomized Tor relay. Each relay decrypts the encryption level to open the next relay in the chain to pass the remaining encrypted data to it. The last relay removes the deepest encryption level and sends the original data to its destination without revealing or knowing the original IP address. Because communication jumps are partially hidden with every hop in the Tor chain, this method eliminates any point at which peers can be identified via network surveillance based on source and destination information [3].

The main product of the Tor project is the Tor Browser implemented by Steven J and Murdoch by the year 2008, January. Sir Bundle. Modified Mozilla Firefox ESR web browser includes Tor browser, Tor button, Tor launcher, No script, HTTPS Anywhere Firefox extensions, Tor and Proxy. Users can play torrent media of an onion browser from removable media [4, 5]. It can run on Microsoft Windows, Mac OS or Linux. The Tor browser automatically starts the Tor background process and routes traffic through the Tor Network. At the end of a session, the browser will delete sensitive privacy data such as HTTP cookies and browsing history [6].A git hub collection is maintained using links to versions which are hosted on other domains to allow downloads from places where access to the Tor project URL can be hijacked or blocked.

Tor installation steps on linux machine

- tar -xvJf tor-browser-linux32-3.6.2_*LANG*.tar.xz – Extracting the Tor Package (32-bit version)
- tar -xvJf tor-browser-linux64-3.6.2_*LANG*.tar.xz – Extracting the Tor Package (64-bit version)
- cd tor-browser_*LANG* – Switching to Tor browser directory
- ./start-tor-browser – Running of Tor browser bundle

Tor Usages

It allows network users to access the Internet, chat and send instant messages anonymously and is used by various people for licensing and illegal purposes. For example, criminal enterprises, activism groups, and law enforcement sometimes use simultaneous investigations. The network is also used for illegal activities. This may include protecting or censoring confidentiality and distributing material about child abuse, drug trafficking or malware, Weapon dealings etc. [7, 8].

Tor Architecture

Tor circuits allow cells to exchange data between nodes without revealing more information than the data sender and receiver. This anonymity is maintained even when channels change every minute to reduce vulnerability to traffic analysis. To save time building circuits at each step, the circuits are pre-created and saved by the proxy. This circuit change is like going to and from your favorite store in town, but each time you take a different route. Channel creation is one of the main features of previous versions of low latency anonymous networks. The chaining methodology is an incremental process that uses a modified onion encryption scheme [9]. Unlike the onion circuit described by Kamenish and Lysyanskaya, Tor iteratively generates circuits that connect each link to each other[10]. Fig.1 depicts the basic functioning architecture model of Tor based network communication.

First, the proxy, node A, sends the creation cell to the first node in its path, node B, which contains the first half of the diffie-hellman handshake key. Then, because a key will be used to connect between A and B, A chooses a different key to use as the chain identifier between the two. B will then respond with a generated cell containing the second half of the diffie-hellman handshake, as well as a negotiated key: the hash of the first and second halves of the DH handshake. It establishes a rudimentary scheme containing A and B. These nodes can now communicate with relay cells, all of which are encrypted with their agreed handshake key [15, 16]. To extend this scheme, A sends B an extended cell containing the address of the next requested node, C and A's DH confirmation key encrypted with a key between A and B. B places this key, as well as an unused identification scheme, inside the payload of the new cell to create C. C sends back his handshake key DH, as well as the key between B and C. B then sends the extended relay cell back to A, which contains the key negotiated between B and C. extended the circuit with one. Thus, each node has a key to communicate with each node in front of it and with the node immediately behind it. This process can be repeated recursively.

The relay cells can now be encrypted and decrypted with each subsequent link. A and B use an unencrypted link, which means that if an attacker controls A or B, then they will have enough information to interpret the request. B is an input node because it accepts cells from A. The last node in the chain that connects to the final destination is the output node. Nodes that do not have direct access to their final destination (except the last one) are intermediaries that go only to other Tor nodes. All these connections are securely encrypted [11]. The most common exit policy is restricted exit, which blacklists services and web addresses that are known to be illegal. Through a secure and repetitive process of building a diagram between a proxy server and a destination, Tor successfully bypassed the circle by revealing any information about the proxy user, provided that the user did not include separate information about themselves or their device [14]. The above figure 2, shows that Tor cell architecture on data communication in Dark Web.

Intrusion Attacks

Intrusion attacks are the new techniques handled by hackers. In the earlier stages, spam-based attacks have occurred on the communications. Due to advanced researches and tools, those spam-based attacks have been controlled. Now, cyber criminals started to intruding the victim's system and data via their IP addresses and open ports of the system. To accomplish this task, they use "Dark Web" environment and they performing intrusions as an anomaly-based entry. Multiple mode of intrusions is possible in this "Dark Web" attacking mode. They are as follows;

1. Network Intrusion
2. Anomaly Based Intrusion
3. Host Based Intrusion
4. Signature Based Intrusion

The above listed intrusions are the few techniques used by an attacker to attack the victim's system. They may target a single host system, Network connected system by the means of unidentified intrusions and the misused signatures [12, 17].

Benchmark Dataset and Feature Selection

For intrusion detection system, researchers are working with numerous datasets. In the beginning, Darpa has created dataset for the local networks to identify the abnormal parameters. Shervin et.al, had a review about the dataset on the network for the classification of normal and abnormal based on two broad categories as Rule based and Machine learning based process [13]. For a first model they have used known pattern for segregating the datasets and this works only on the known rules. Second model works on the machine learning algorithms such as Support vector machine (SVM), Markov model (MM), Naïve bayes model to separate the data features for classifications. Those classifiers are bringing the new dataset on the trained model to train the test dataset [19, 20]. Mostly these classifiers are used to predict the expected outcomes. The following table 1 shows that KDD99 dataset features, which supports the proposed model to identify intrusive attacks. It contains 41 different features belonging to 4 classes of attacks as follows; 1) Normal 2) User to root (U2R) 3) Local to remote (L2R) and 4) Probe [18].

The reduction of data happens on feature selection process and feature extraction process using machine learning along with linear discriminant feature analysis.

Procedure to classifying an intrusive dataset

Capturing *input and output traffic*
Categorize *traffic parameters*
Check *for valid parameters*
#Compare with labeled classifiers and their parameters
If *(Captured parameters == Labelled items)*
    Move *to concerned cluster*
Else
#Check for response time
    If *(Response time == Threshold value)*
#Check for resources accessibility
        If *(Resources Unavailable == True)*
#Check for malfunction
            If *(System's malfunction == False)*
Accept the packet
Else
    Move *parameters into new cluster*
    Update *new cluster then*
Make *a new cluster as intrusive*

The following equation (1) involved in reduction of dataset from the original data.

$$MI\ (X:Y)\ =\ \sum_{i=1}^{n}\sum_{j=1}^{n} P(X(i),Y(j))\ .\ log\left(\frac{P(X(i),Y(j))}{P(X(i)).P(Y(j)))}\right) \quad (1)$$

Where,
X, Y are the two different datasets in the network traffics.
In the real time payload option, overall parameters are represented in 256 dimensional values. The correlated feature set values are represented by the following equation (2),

$$\sum_{j=1}^{256} f_i = 1, \qquad Q = [f_1 f_2 f_3 \ldots . f_{256}]^T \tag{2}$$

In this proposing research works, multi-tier model is the important role of event analysis. Therefore, all the collected input values are analyzed by principal component analysis to extract the important features from the tier one. The covariance of the obtained data values and their eigen values and eigen vectors have been arranged in a diagonal matrix format and the same has represented by the following form,

$$C_Q = \frac{1}{m-1} Q_{sh} {Q_{sh}}^T \tag{3}$$

Mahalanobis Distance Map (MDM), is used in this model to find the hidden associated feature vectors and their values of the newly generated dataset from the traffics. This MDM for the data values is derived as follows,

$$\Sigma_a = (x_a - \mu)(x_a - \mu)^T \; (1 \leqslant a \leqslant K_{final}) \tag{4}$$

$$d_{a,b} = \frac{(x_a - x_b)(x_a - x_b)^T}{\Sigma_a + \Sigma_b} \quad (1 \leqslant a, b \leqslant K_{final}) \tag{5}$$

Covariances or the distance value of the newly coming dataset is calculated as a threshold value of the normal dataset and the same has used to find and compare with incoming dataset to analyse them against normal and abnormal vectored parameters to classify them. This weight or threshold value calculation done through the below equation (6).

$$w = \sum_{a,b=1}^{K_{final}} \frac{\left(d_{obj(a,b)} - \overline{d_{nor(a,b)}}\right)^2}{{\sigma^2}_{nor(a,b)}} \tag{6}$$

From GATECH dataset of the HTML related items, Get() and Post() queries are analysed to fetch the URL related information and the form submission related information to process them for finding their originalities. The reduced or the selected features are works for the same to identify the abnormal data items based on the specified threshold values.

Implementation Procedure for Shadow Link Processor

The following steps are showing that implementation of Shadow link processor algorithm for web crawler's log event analysis.

Shadow – Link Process: Implementation Procedure

SeedURL Key Phrase (SU)
Searchfor Web Page (WP)
Retrieve Web Pages for Reference Links (WP(L_i)),

$$\boldsymbol{Web_{Page}(SU) = WP(L1) + WP(L2) + \cdots + WP(L_{n-1})} \tag{7}$$

ParseWeb Page code to extract Outbound URLlinks (WP(L_i)),

$$\boldsymbol{Web_{Page}(SU) = \frac{WP(L1)}{OL(L1)} + \frac{WP(L2)}{OL(L2)} + \cdots + \frac{WP(L_{n-1})}{OL(L_{n-1})}} \tag{8}$$

Check *for Web Page Freshness/Age* to assign score and rank value for concern pages,

$$\boldsymbol{Page_{Rank} = IAV + \sum_{PC=0}^{N} \frac{PRV}{No.of\ OL}} \tag{9}$$

Where,

N=Number of data values
IAV = Initial Age Value,
PC = Number of Pages Visited,
PRV = Page Rank Value,
OL = Outbound Links

Standardize *recorded* data set for classification (SD)

$$SD = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2} \quad (10)$$

Where,

N=Number of data values
(xi – x ') = Mean value for the previous data values

Perform *linear separation* of data set,

$$W^T x + b \geq 1 \; and \; W^T x + b \leq 1 \quad (11)$$

Where,

W = Weight (vector value)
b = Bias value (Scalar value)

Look *for Misclassification Error*,

$$M_{err} = min\frac{1}{2}\|w\|^2 + C\sum_{i=1}^{N}\xi i \quad (12)$$

Where,

$min\frac{1}{2}\|w\|^2 = Hypher\ plane\ for\ Linear\ Classification$
$\xi = Slack\ Variable$

If (Outbound links==existed)***then***
returnOL as Web Page
Else
update index values for Outbound Links
While (Visited OL==Null)
Updatethe index checking process
Continuetill end of all the links in crawling

RESULT AND DISCUSSION

The proposed research model is working under the concept of multi-tier intermediate web crawling operations, all the data are targeted towards the HTML based query instructions. This dataset reduction provides 99.97% of true positive and 1.99% false positive along with 98.01% true negative values and the false negative value as 0.03%. The table 2, showing performance metrics values of the Niave Bayesian and Logistic Regression classifying model on training and testing dataset. On every data transaction, end devices used to check for error in the received data. Fig. 3 (a) and (b), showing that unverified checksum value of every transferring data on the time duration of every second and ten second interval time. This checksum value is an important parameter to verify the data modifications on data transactions. Whenever network data transaction occurs, end devices used to share their sensitive data to the opposite system in the encrypted format. To identify sequences of all the encrypted information, both end systems have the checksum option to verifying the received data. In which, those system IP addresses, Port numbers, Data length, Payload information etc., where added and the same will be verified to identify the reliability of the transferred data. The figure 3 (a) and (b) are diagram representing the TCP-Checksum errors on the given input URL.

CONCLUSION

This paper talks about "Dark Web" and "Intrusion Attacks along with Detection Procedure". The above-mentioned procedure is a generic method to identify the abnormal parameters of an input and output network traffics on the communication medium. If all the incoming and outgoing parameters were analyzed in the given procedure, simply every user can restrict minimum of 90% of intrusion type of attacks. When Classification procedure and attacking parameter identification will be automated then the performances of an intrusion detection system will be increased a lot. As a future direction, deploying an automated IDS will be helping the victims to protect their sensitive data and increases the system's security functions.

REFERENCES

1. Beshiri, A. and Susuri, A. (2019) Dark Web and Its Impact in Online Anonymity and Privacy: A Critical Analysis and Review. *Journal of Computer and Communications*, **7**, 30-43. doi: 10.4236/jcc.2019.73004.
2. Y. Y. Aung and M. M. Min, "An analysis of random forest algorithm based network intrusion detection system," 2017 18th IEEE/ACIS International Conference on Software Engineering, Artificial Intelligence, Networking and Parallel/Distributed Computing (SNPD), 2017, pp. 127-132, doi: 10.1109/SNPD.2017.8022711.
3. Termanini, Rocky (2017). The Nano Age of Digital Immunity Infrastructure Fundamentals and Applications: The Intelligent Cyber Shield for Smart Cities. CRC Press. pp. 210–211. ISBN 978-1-351-68287-9. LCCN 2017053798.
4. Ball, James; Schneier, Bruce; Greenwald, Glenn. "NSA and GCHQ target Tor network that protects anonymity of web users". 4, October 2013.
5. https://web.archive.org/web/20140623203436/https://www.torproject.org/projects/torbrowser.html.en
6. Murdoch, Steven J. (30 January 2008). "New Tor distribution for testing: Tor Browser Bundle". tor-talk (Mailing list).
7. https://2019.www.torproject.org/about/overview.html.en
8. Jardine, Eric; Lindner, Andrew M.; Owenson, Gareth (15 December 2020). "The potential harms of the Tor anonymity network cluster disproportionately in free countries". Proceedings of the National Academy of Sciences. 117 (50): 31716–31721. doi:10.1073/pnas.2011893117. ISSN 0027- 8424.
9. https://medium.com/@gkaufmann/the-tor-architecture-and-its-inherent-security-implications-61f45fd42b01#:~:text=The%20architecture%20of%20the%20Tor,reduce%20susceptibility%20to%20traffic%20analysis.
10. Camenisch, Jan, and Anna Lysyanskaya. A Formal Treatment of Onion Routing. cs.brown.edu/ people /alysyans/papers/cl05.pdf
11. Roger Dingledine, Nick Mathewson, Paul Syverson, "Tor: The Second-Generation Onion Router", June, 2004.
12. S. Ponmaniraj, Dr. Tapas Kumar, Dr. Amit Kumar Goel. "Web Intrusion Detection System: Identification Of Web Server Reliability On Internet Connections Through Log File Pre-processing". JCR. 2020; 7(18): 1971-1978. doi:10.31838/jcr.07.18.247
13. Shervin Minaee, NalKalchbrenner, Erik Cambria, NarjesNikzad, MeysamChenaghlu, and Jianfeng Gao. 2020. Deep Learning Based Text Classification: A Comprehensive Review. 1, 1 (January 2020), 43 pages. https://doi.org/10.1145/nnnnnnn.nnnnnnn
14. Mittal, S.; et al.: Cybertwitter: using twitter to generate alerts for cybersecurity threats and vulnerabilities. In: Proceedings of the 2016 IEEE/ACM International Conference on Advances in Social Networks Analysis and Mining. IEEE Press (2016) 18.
15. Mallik, Avijit& Ahsan, Abid & Shahadat, Mhia& Tsou, Jia-Chi. "Man-in-the-middle-attack: Understanding in simple words". 3. 77-92. 10.5267/j.ijdns.2019.1.001, 2019.
16. G. Helmer. Intelligent Multi-Agent System for Intrusion Detection and Counter-measures. PhD dissertation, Iowa State University, Computer Science, Ames, IA, 2000.

17. S. Nazah, S. Huda, J. Abawajy and M. M. Hassan, "Evolution of Dark Web Threat Analysis and Detection: A Systematic Approach," in IEEE Access, vol. 8, pp. 171796-171819, 2020, doi: 10.1109/ACCESS.2020.3024198.
18. Creech G, Hu J. Generation of a new IDS test dataset: Time to retire the KDD collection. In: 2013 IEEE Conference on Wireless Communications and Networking (WCNC), 2013; p. 4487-4492.
19. Ankit Thakkar, Ritika Lohiya, "A Review of the Advancement in Intrusion Detection Datasets", International Conference on Computational Intelligence and Data Science (ICCIDS 2019), Issue 167, PP 636-645, 10.1016/j.procs.2020.03.330
20. Rana Aamir Raza Ashfaq, Xi-Zhao Wang, Joshua Zhexue Huang, Haider Abbas, Yu-Lin He, Fuzziness based semi-supervised learning approach for intrusion detection system, Information Sciences, Volume 378, 2017, Pages 484-497, ISSN 0020-0255, https://doi.org/10.1016/j.ins.2016.04.019.

Table 1. KDD99 Dataset Feature

S. No	Feature	S. No	Feature	S. No	Feature	S. No	Feature
1	Duration	12	Logged_in	23	Count	34	Dest_host_same_Serv_rate
2	Protocol_type	13	Num_compromised	24	Serv_count	35	Dest_host_diff_ Serv_rate
3	Service	14	Root_shell	25	Serror_rate	36	Dest_host_same_src_port_rate
4	Flag	15	Su_attempted	26	Serv_serror_rate	37	Dest_host_Serv_diff_host_rate
5	Src_bytes	16	Num_root	27	Rerror_rate	38	Dest_host_serror_rate
6	Dest_bytes	17	Num_file_creations	28	Serv_rerror_rate	39	Dest_host_Serv_serror_rate
7	Land	18	Num_shells	29	Same_Serv_rate	40	Dest_host_rerror_rate
8	Wrong_fragment	19	Num_access_files	30	Diff_Serv_rate	41	Dest_host_rerror_rate
9	Urgent	20	Num_outbound_cmds	31	Serv_diff_host_ rate	Not Applicable	
10	Host	21	Is_hot_login	32	Dest_host_count	Not Applicable	
11	Num_failed _logins	22	Is_guest_login	33	Dest_host_Serv_ count	Not Applicable	

Table 2. Performance Metrics Values on Normal and Abnormal Classification Using Naïve Bayesian and Logistic Regression Model

	Methods	Naïve Bayesian Model		Logistic Regression Model	
	Performance Metrics	**Anomaly**	**Normal**	**Anomaly**	**Normal**
Evaluating Model	***Precision***	0.95	0.88	0.96	0.95
	Recall	0.85	0.96	0.94	0.97
	F1-Score	0.9	0.92	0.95	0.96
	Support	8245	9389	8245	9389
Validating Model	***Precision***	0.94	0.88	0.96	0.95
	Recall	0.85	0.95	0.94	0.97
	F1-Score	0.89	0.92	0.95	0.96
	Support	3498	4060	3498	4060

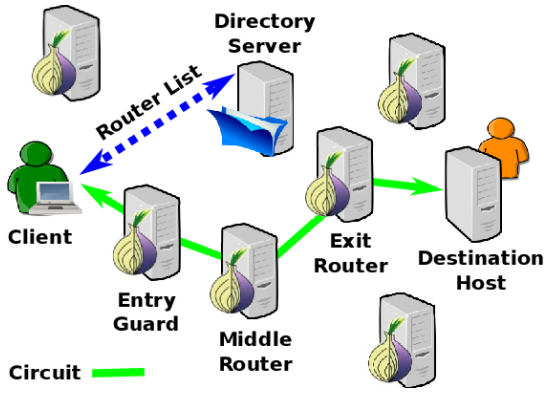

Figure 1. Tor System Architecture Model

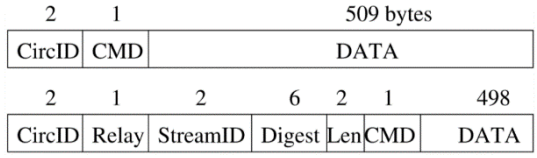

Figure 2.Tor Cell Architecture

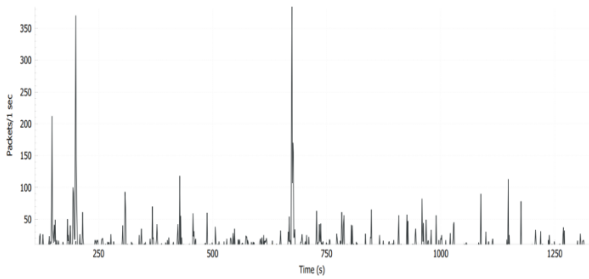

Figure 3 (a). TCP Errors - Checksum Unverified Per Second

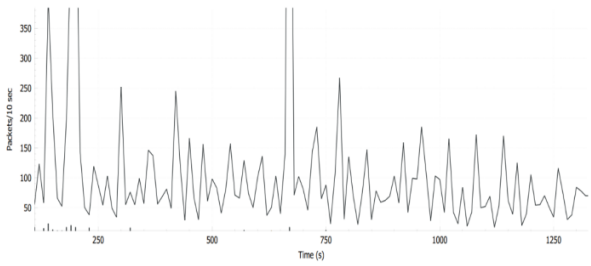

Figure 3 (b). TCP Errors - Checksum Unverified Per 10 Second

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RESEARCH ARTICLE

An Semi Δ - Open Sets in Minimal Topological Spaces

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ABSTRACT

In this paper, we introduce new classes of m-semi Δ-open sets in minimal spaces. It is obtained by generalizing m- Δopen sets in the same way that m-semiopen were generalized m-open sets. We study some properties of m-semi Δ-open sets in minimal spaces. We also define the m-semi Δ-interior and the m-semi Δ-closure of a set A in a space (X, m_x).
Mathematics Subject Classification: 54A05, 54A20, 54C08, 54D10

Keywords: m-Δ-open sets, m-Δ-closed sets, m-semi Δ-open sets, m-semi Δ-closed sets, m-semi Δ-interior and m-semi Δ-closure.

INTRODUCTION

In 1963, Levine [6] defined semi-open sets, which are weaker than open sets in topological spaces. In the years 2001 and 2003, F.Nakaoka and N. Oda[3,4] introduced and studied minimal open (resp. minimal closed) sets which are subclasses of open (resp. closed) sets. The complements of minimal open sets minimal closed sets respectively. In [7], Popa and Noiri introduced the notion of minimal structure which is a generalization of a topology on a given nonempty set and they introduced the notion of m-continuous function as a function defined between a minimal structure and a topological space. The concept of minimal structures (briefly, m-structure) were widely studied by Popa and Noiri [7] in 2000. In this paper, we introduce and study the notion of m-semi-Δ-open sets and it's defined between a minimal structure and a topological space.

Preliminaries

Definition: 2.1 [1,2, 5]

A subset $A \subseteq X$ is said to be semi-open if there exists $O \in \tau$ such that $O \subseteq A \subseteq cl(A)$. A is semi-open then if and only if $A \subseteq cl(int(A))$. The complement of a semi-open set is said to be semi-closed.

Definition: 2.2 [8]

A subset A of a space X is said to be Δ-open if $A = (B - C) \cup (C - B)$, where B and C are open subsets of X. The complement of a Δ-open set is said to be Δ- closed. The notions of Δ-interior and Δ- closure of a set A in X are defined analogue to interior and closure of a set A in X. Let (X, τ) be a topological space and let $A \subseteq X$. Then the closure of A and the interior of A will be denoted by cl(A) and int(A), respectively.

Definition: 2.3 [7]

A subfamily $m_x \subset$ P(X) is said to be a minimal structure on X if $\phi, X \in m_x$. The pair (X, m_x) is called a minimal space (or an m-space). A subset A of X is said to be m-open if A $\in m_x$. The complement of an m-open set is called m-closed set. We set mInt(A)= $\bigcup\{U : U \subset A, U \in m_x\}$ and mCl(A) =$\cap${F: A $\subset$ F, X − F $\in$ m_x}.

Definition: 2.4 [7]

Let (X, m_x) be a space with a minimal structure m_x and A ⊆ X. Then

(1) mInt(A) ⊆ A.
(2) If A ⊆ B, then mInt(A) ⊆ mpInt(B).
(3) A is m-open iff mInt(A) = A.
(4) mCl(X − A) = X − mInt(A) and mInt(X − A) = X − mCl(A).

Definition: 2.5 [7]

A subset A of a minimal space (X, m_x) is said to be

(a) regular m- open set if $A = m\text{-}Int(m\text{-}Cl(A))$.

(b) m-α -open if $A \subset m\text{-}Int(m\text{-}Cl(m\text{-}Int(A)))$.

(c) m-semi open if $A \subset m\text{-}Cl(m\text{-}Int(A))$.

(d) m-preopen if $A \subset m\text{-}Int(m\text{-}Cl(A))$.

The complement of a regular m-open (m-α -open, m-semiopen, m-preopen) set is called a regular m-closed (m-α -closed, m-semiclosed, m-preclosed) set.

Definition: 2.6 [7]

A minimal space (X, m_x) has the property [U] if "the arbitrary union of m-open sets is m-open". (X, m_x) has the property [***I***] if "the any finite intersection of m-open sets is m-open" .

m- Semi Δ-Open sets

Definition: 3.1

A subset S of a space (X, m_X) is said to be m-semi Δ-open if $S = (A - B) \cup (B - A)$, where A and B are m-semiopen sets in X.

Theorem: 3.2

Every m- Δ-open set as also every m-semiopen set is m-semi Δ-open. But the opposite of the implications need not to be true.

Example: 3.3

Let $X = \{a, b, c\}$ and $m_X = \{X, \phi, \{b\}\}$.Then {a} is m-semi Δ-open. But is neither m- Δ-open nor m-semi-open.

Theorem: 3.4

If S is a m-semi Δ –open subset of a space(X, m_X), then there exists a m- Δ-open set A such that $O \subseteq S \subseteq mcl(A)$.

Proof

S is m-semi Δ –open, there exist m-semi-open sets A and B such that $S = (A - B) \cup (B - A)$. Now, A and B are m-semi-open sets, it follows that there exist m-open sets U and V in X such that $U \subseteq A \subseteq$ mCl(U) and $V \subseteq B \subseteq$ mCl(V). This implies that

$(U - V) \cup (V - U) \subseteq (A - B) \cup (B - A) \subseteq$ [(mCl(U)) –(mCl(V))] ∪ [(mCl(V)) – (mCl(U))] ⊆ [mCl(U – V)] ∪ [mCl(V – U)] = mCl[(U – V) ∪ (V-U)].

Theorem: 3.5

If O is m-open and S is m-semi Δ-open in a space (X, m_X), then O∩S is m-semi Δ-open in X.

Proof

$O \cap S = O \cap [(A - B) \cup (B - A)]$. Where A and B are m-semi-open sets in X.

$= [O \cap (A - B)] \cup [O \cap (B - A)]$

$= [(O \cap A) - (O \cap B)] \cup [O \cap B) - (O \cap A)]$

Which is m-semi Δ-open since $O \cap A$ and $O \cap B$ are m-semi open sets.

Theorem: 3.6

A subset A of a space (X, m_X) is m-semi Δ-open if and only if $A \subseteq$m-Cl(Δ-m-Int(A)).

Proof: Let A be a m-semi Δ-open subset of X. Then there exists a m- Δ-open set U such that $U \subseteq A \subseteq$mCl(U). But $U =$ Δ-mInt(U)⊆ Δ-mInt(A) and so m$cl(U) \subseteq$ mCl(Δ-mInt(A). Thus $A \subseteq$mCl(U) ⊆ mCl(Δ-mInt(A)). Now, A⊆ mCl(Δ-mInt(A). suppose put $U =$ Δ-mInt(A). Then U is m-Δ-open with $U \subseteq A \subseteq$ mCl(Δ-mInt(A)). Hence $U \subseteq A \subseteq$ mCl(U) and A is m-semi Δ-open.

Theorem: 3.7

If $\{A_\alpha : \alpha \in I\}$ is a family of m-semi Δ-open subsets of (X, m_X), then $\cup_{\alpha \in I} A_\alpha$ is m-semi Δ-open.

Proof

For each $\alpha \in I$, there exists a m-semi Δ-open set U_α such that $U_\alpha \subseteq A_\alpha \subseteq$m $cl(U_\alpha)$ Now $\cup_{\alpha \in A} A_\alpha \subseteq \cup_{\alpha \in I} A_\alpha \subseteq \cup_{\alpha \in I}[mcl(U_\alpha)] \subseteq mcl(\cup_{\alpha \in I} U_\alpha)$. Then $\cup_{\alpha \in I} A_\alpha$ is m-semi Δ-open.

Definition: 3.8

Let (X, m_X) be a minimal space and let $A \subseteq X$. Then the union of all m-semi Δ-open sets contained in A, denoted by $s\Delta\ (mint(A))$, is called the m-semi Δ-interior of A. It is clear that m$int(A) \subseteq s$[mInt(A)] ⊆ $s\Delta$ [mInt(A)] for any subset A of X. Recall that sm-int(A) is the m-semi-interior of $A \subseteq X$.

Theorem: 3.9

Let A be a subset of a space (X, m_X). Then $s\Delta$ -m$int\ (A)$ = $A \cap$ m-Cl(Δ-mInt(A)).

Proof

$A \cap$ mCl(Δ-mInt(A))⊆ mCl(Δ -mInt(A ∩ mInt(A))) ⊆ mCl(Δ -mInt(A ∩mCl(Δ -mInt(A))). Thus $A \cap$ mCl(Δ -mInt(A) is m-semi Δ-open set contained in A. Hence ∩ mCl(Δ-mInt(A))⊆ $s\Delta$ -m$int\ (A)$. On the other hand since $s\Delta$ -m$int\ (A)$ is a m-semi Δ –open set, we have $s\Delta$ -m$int\ (A)$ ⊆mCl(Δ-mInt(sΔ-mInt(A))) ⊆ mCl(sΔInt(A)). $s\Delta$ -m$int\ (A)$ = $A \cap$ mCl(Δ-mInt(A)).

Theorem: 3.10

Let (X, m_Y) be a subspace of a space (X, m_X) and let $A \subseteq Y$. If A is m-semi Δ-open in X, then A is m-semi Δ-open in Y.

Proof

A is m-semi Δ −open in X, there exists a m- Δ −open set U in X such that $U \subseteq A \subseteq$ mCl(U). Then $U = U \cap Y \subseteq A \subseteq$ mCl(U) $\cap Y$ =mCl$_Y$(U). Thus A is m-semi Δ-open in Y.

Theorem: 3.11

Let Y be a m- Δ-open set in a space (X, m_X). If $A \subseteq Y$ and A is m-semi Δ-open in Y, then A is m-semi Δ-open in X.

Proof

Let A be m-semi Δ −open in Y. Then there exists a m-Δ-open subset U of (Y, m_Y). such that $U \subseteq A \subseteq$ mCl$_Y$(U). Since Y is m- Δ-open in X, therefore, U is m-Δ-open in X and $U \subseteq A \subseteq$ mCl$_Y$(U) $\subseteq$ mCl(U). Hence A is m-semi Δ-open in X.

Definition: 3.12

A subset A of a space (X, m_X) is said to be m-semi Δ-closed iff X$\in A$ is m-semi Δ-open.

Remark: 3.13

Since all m-open sets are m-semi Δ-open, it follows that all m-closed sets are m-semi Δ-closed.

Definition: 3.14

Let A be a subset of a space (X, m_X) , then the m-semi Δ-closure of A, denoted by $s\Delta mcl(A)$, is defined as the intersection of all m-semi Δ −closed subsets of X containing A.

Remark: 3.15

$s\Delta mcl(A) \subseteq smcl(A)$ for any $A \subseteq X$.

Theorem: 3.16

A subset F of a space (X, m_X) is m-semi Δ −closed if and only if m- $int(\Delta\, mcl\,(F)) \subseteq F$.

Proof

Obvious.

Theorem: 3.17

If A is a subset of a space (X, m_X) then $s\Delta m\, cl(A) = A \cup mint(\Delta mcl(A))$.

Proof

$mint\left[\Delta mcl\left(A \cup mint(\Delta\, mcl\,(A))\right)\right] \subseteq mint[\Delta\, mcl(A \cup mcl(A))]$.

$= mint\,[\Delta\, mcl\,(A)] \subseteq A \cup mint[\Delta\, mcl\,(A)]$.

Thus by theorem 8, $A \cup mint[\Delta\, mcl\,(A)]$ is a m-semi Δ− closed set containing A and so $s\Delta mcl(A) \subseteq A \cup mint(\Delta\, mcl(A))$.

On the other hand, since $s\Delta mcl(A)$ is m-semi Δ− closed, therefore, $mint[\,\Delta\, mcl(s\,\Delta\, mcl\, A))] \subseteq s\,\Delta\, mcl\,(A)$. Hence $mint[\Delta\, mcl\,(A)] \subseteq mint[\Delta mcl($ $s\Delta mcl(A) = s\Delta mcl(A)$ and consequently $A \cup mint\,(\Delta\, mcl\, A) \subseteq s\Delta mcl(A)$. Thus $s\Delta mcl(A) = A \cup mint\,(\Delta\, mcl\, A)$.

Theorem: 3.18

Let A is a subset of (X, m_X) then s$\Delta mcl\,(A) \subseteq s\, mcl\,(A) \cap \Delta mcl(A)$.

Proof

s $\Delta mcl\,(A) = A \cup mint\,(\Delta\, mcl\,(A))$

$\subseteq mint\,(mcl(A)) = s\, mcl\,(A)$

Also, $s\Delta mcl(A) \subseteq A \cup \Delta\, mcl(A) = \Delta mcl\,(A)$. Therefore, $s\Delta mcl\,(A) \subseteq smcl(A) \cap \Delta mcl\,(A)$.

Remark: 3.19

In Theorem (3.18) equality conditions does not satisfied.

Example: 3.20
Let $X = \{a, b, c\}$ and $m_X = \{X, \phi, \{a, b\}, \{b\}\}$. Then $smcl(\{a, b\}) = X = \Delta mcl(\{a, b\})$ but $s\Delta mcl(\{a, b\}) = \{a, b\}$.

Theorem: 3.21
If F is m-closed and S is m-semi Δ −closed in a space (X, m_X), then $F \cup S$ is m-semi Δ −closed.
Proof
$X - F$ is m-open and $X - S$ m-semi Δ −open. Then by Theorem 2, $X - F \cap X - S$ is m-semi Δ −open. That is $X -(F \cup S)$ is m-semi Δ-open. Hence $(F \cup S)$ is m-semi Δ −closed.

Theorem: 3.22
Let (X, m_X) be a minimal space and $A \subseteq X$. Then
(a) A is m-semi Δ-open iff A = $s\Delta imnt\ (A)$.
(b) A is m-semi Δ-closed iff A = $s\Delta mcl\ (A)$.
(c) $s\Delta\ mint(X - A) = X - s\Delta mcl(A)$.
(d) $s\Delta mcl(X - A) = X\ s\Delta\ mint\ (A)$.

Theorem: 3.23
If A is a subset of a space (X, m_X), then the following are equivalent.
(a) A is a m-dense subset of X.
(b) $s(\Delta mcl\ (A) = X$.
(c) If F is a m-Δ-closed subset of X and $U \subseteq F$, then F = X.
(d) For any non-empty m-Δ −open subset S of $(X, m_X), S \cap A \neq \phi$.
(e) $s\Delta\ mint\ (X - A) = \phi$.
Proof
Obvious.

REFERENCES

1. S.G. Crossley and S.K. Hildebrand: Semi-closure, Texas J. Sci. 22(1971), 99-112.
2. P. Das: Note on some applications on semi-open sets, progress. Math. 7(1973), 33-44.
3. F. Nakaoka and N. Oda: Some applications of minimal open sets, *Int. J.Math. Math. Sci.* 27- 8, 471-476 (2001).
4. F.Nakaoka and N.Oda: On minimal closed sets, Proceeding of Topological Spaces Theory and its Applications, 5,19-21 (2003).
5. T.M. Nour: A note on some applications of semi-open sets, Internat. J. Math. Sci. 21(1) (1998), 205-207.
6. N. Levine: Semi-open and semi-continuity in topological spaces, Amer. Math. Monthly,
7. 70(1963), 36-41.
8. V.Popa and T.Noiri: On M-Continuous Functions, Anal. Univ. "Dunarea de Jos" Galati, Ser. Mat. Fiz. Mec. Tecor., 18(23)(2000), 31-41.
9. M. Veera Kumar: On Δ-open sets in topology, to appear.

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RESEARCH ARTICLE

Hall Effect on MHD Flow of a Visco-Elastic Fluid through Porous Medium Over an Infinite Vertical Porous Plate with Heat Source

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ABSTRACT

In this article, we've studied the unsteady movement of an incompressible viscoelastic fluid (Walter's B) in conjunction with the heat transfer near an oscillating plate that incorporates a porous channel taking the current into consideration. When the governing equations are broken into small segments and the problem has a small elasticity, a perturbation procedure is applied to each segment. Main, secondary, and transverse velocity, have been analytically and computationally studied using graphs as well as with relation to the skin-friction data, and mathematical functions.

Keywords: Hydromagnetic, Viscoelastic, Hall Effect, Porous medium, Slip-flow regime, heat generation.

INTRODUCTION

Applied by a vast numbers of researchers to this slip-flow model is the idea that several different values are approached when using differing products and procedures, it's because of this broad range of applications that people have seen. During this modern times of complex technology and rapid industrialization, societal and global change, knowing how to maximise the flow of information becomes ever more critical. Any particles located on a surface move at the same speed with respect to the surface (it no longer matters whether it is fluid or solid) The electron on the particle's surface has a tangential velocity that can be calculated; it skips around the surface. One of the assumptions behind expandable sets is that slip must be considered, and cannot be overlooked. Often known as “thin film hydrophobic coat on moving plate", "nano-membrittainleylic hydrophobic coating of the body", is the slippage phenomenon at the solid boundary seen in micro channels and thin oil films of light oils or light

hydrophobic coatings applied to items, where the surface is treated with monolayer hydrophobic coating. Free convection past a semi-infinite semi-infinite semi-conducting, viscous fluid surface with an aligned magnetic and latent heat mechanism while passing over an infertile, compared to all authors in Alam et al. [2] which compared free convection in the influence and mass transfer over a surface on electrically conducting and nonviscous viscous in the presence of a magnetic flow. Kesava and Venkavulu studied unsteady Couette radiative mass transfer in semi-infinite vertical channel with heat absorption, Chamkha explored convective heat and chemical reaction in vertical porous plates. The invention of impulsively initiated plates to deal with expansion and resistance-visc heat and mass transfer has been classed as the vertically limitless plate inelastic-viscs heat and mass tramp fluid they found (1982) that Derek, et al. (1982) researched the apparent fluid slippage at the hydrophobic channels, meaning, is the presence of a thin layer of lubricant on the outer surface of another item or on the working surface of a system which there is apparent slip of a lubricant (sliding over another), where the surfaces are oiled to reduce friction and similar studies found that heat and mass transfer occur in a viscoelastic fluid as a volumically driven horizontal flow through a catalytic reactor with chemical reaction to be inconsistent with an assumed non-elasticity The authors Hady and associates [8] studied the issue of free convection through vertically-embedded wavy channels embedded in conducting media that are concentrated or actively absorbing heat, particularly in the presence of internal generation of said to be fully or distribution of an impact for moisture, for his doctoral dissertation. Hossein and his colleagues [9] investigated the issue of natural convection occurring in vertical laminar fashion on a wavy surface with a surface that generates or absorbs heat uniformly. in a paper presented in "Flow of a viscoel fluid between coaxial rotating discs at uniform pressure or pressure injection," "Flow of a viscoel fluid between coaxial rotating discs,"

The most numerous instances in which convective flows provide both thermal and mass transfer to occur with a magnetic forces under the control of a reaction is in the research and engineering divisions are in the transport applications. This phenomenon has significant applications in the drying of solvents, the fabrication of metal vapour deposition, chemical vapour deposition of surfaces, and cooling of nuclear reactors, as well as in the power and refrigeration industries. Consequently, on account of natural convection, temperatures becoming lower, density increasing is a lot. A variation in temperature may cause certain concentrations to expand or contract. For example, flow is caused by changes in temperature as well as in the environment as well as a difference in concentration in water vapour concentrations in the atmosphere. Khalid and Vafai developed an expression for free convection in the unsteady, nonlinear magnetohydrostatic field for periodical and constant velocity, Jain and Gupta examined unsteady unsteady unsteady convection in the flow-expand regime, and Vaimationarity with variable permeability, and constant heat flux, and V for steady state and flux, and Vafai found an expression for unsteady regular magnetohydrodynamic field for constant and periodic flows. Flow over a channel with permeable boundaries having been explored by Makind and Osalusi [see Makind-Osalusi (2011, Chapter 11) for a discussion of slip conditions] slip and concentration tested the idea of flow across porous media by means of both, and Mansour et al. [14] performed the tests of convective micro-micropicity over temperature and concentration. Dong et al. [15] have analysed the slippage in Couette flow with regard to its affect on the plate motion helical rod, with unsteady convective heat and mass transfer in a vertical column with Viscoelastic flow has been covered by Mehmood and Ali in the Mehmood and associates' article 'Progress in Thermal Analysis of Viscoelastic Flow through Helical Rods' and by Mohiddin and associates (or Mohiddin et al.). Because irradiation of unsteady fluid flows may potentially occur in a turbulent shear cases with a heated surface, Srinathuni Lavanya et al. [18] stated that the impact of radiation on the unsteady convection of viscous MHD flows in unsteady shear MHD flow has also to be investigated. two impermeable planar-parallel media. Prasuna et al. [19] studied an unsteady flow of a viscoelastic fluid that flows across a porous medium from two impermeable plates. Using the unsteady viscoel MHD flow from Oldroyd in a flow streamtube apparatus, Rahmann and Sarkar [20] examined unsteady MHD motion in a channel with a rectangular cross-channel sensor system.

Oil, some silicone solution, some stains, as well as certain synthetic polymers, and many of the latest compounds are thinning liquids, although many of the older compounds show different characteristics, in particular viscosity. Due to the aforementioned, the visco-elastic nature of these fluids, there is a good deal of study being done on them. it

has been seen in fluid flowing from a thin plate with an infinite/semi-compressible viscous wall to a thick plate without altering the speed of the movement of the viscous fluid Effervescent fluid flow has caught the interest of scientists and engineers because of its notable use in the movement of oil through porous rocks, the processing of energy from geothermal resources, the production of effluents from landfills, and the flow of solids through porous media for human administration, as well as skin-derived substances. Conventional recovery techniques work for water miscible fluids cannot be used for the separation of crude oil from reservoir rocks as effectively as with natural crude oils. Ground water and runoff issues often affect adsorption and filtration. to Rajagopallis, who expanded the Walter model, which is in the forced convection of a viscoelastic fluid, Rajagari [21]looked the heat transfer. Rajagasthan [22] conducted extensive studies on the electric oscillation of viscoelastic fluids in a saturated porous medium over stretching, with the medium in an oscillatory state. Mallikarjuna Reddy et al. [23] has included unsteady convective viscous free-diffusion past an infinite vertical plates in the scope of results generated by radiation and diffusion on a field visco-porous heat source, and Sharma [24] addressed unsteady viscous unstressed convective fluid flow in an immiscible flow that operates in a transd cyclic regime with periodic heat source application. Via viscoelastic movement, Singh and Oldroyd [25] via viscoelastic (RMP) flow of a dusty fluid in a parallel configuration, Singh and Singh [study MHD flow of a viscoelastic (PA) fluid) between two plates tilted to the horizon] It is not possible to ignore the Hall current's influence on the magnetic field intensity when the strength of the magnetic is high. it is important to learn how the influence of stream flow resistance on the outcome of hydro-dynamic problems is. Rashidic and Kumar [26] investigated the exact solution of an oscillatory MHD flow through a porous medium situated in a revolving channel that is bounded by a porous wall to see whether it exhibited existing blockage. The slip flow regime can also occur in the working fluid containing concentrated suspensions Soltani and Yilmazer [27]. Chenna Kesavaiah et. al. [28] has been studied The results of a chemical reaction on MHD flow in a vertical tube on a porous medium Bhavana and Chenna Kesavaiah [29] has been discussed perturbation solution for thermal diffusion and chemical reaction effects on MHD flow in vertical surface with heat generation, In the experiments described here, unsteady fluid flow was driven past an infinite-porosity vertical plate at a given rate, through a porous media with differing flow generation coefficients were combined with heat generation, and thus a particular emphasis was placed on understanding the effect of those on the unsteadiness.

Mathematical Formulation

We consider the unsteady flow of a viscous incompressible and electrically conducting visco - elastic fluid with oscillating temperature and heat generation taking in to an account. The flow occurs over an infinite vertical porous plate. The x^*-axis is assumed to be oriented vertically upwards along the plate and y^*-axis is taken normal to the plane of the plate. It is assumed that the plate is electrically non-conducting and a uniform magnetic field of strength B_0 is applied normal to the plate. The induced magnetic field is assumed to be negligible so that $\vec{B}(0, B_0, 0)$. The plate is subjected to a constant suction velocity V_0.

The constitutive equations for the theological equation of the state for the visco-elastic fluid (Walter's liquid B') are:

$$p_{ik} = pg_{ik} + p^*_{ik} \quad (1)$$

$$p_{ik} = 2\int_{-\infty}^{t} \psi\left(t - t^*\right) e^{(1)}_{ik}\left(t^*\right) dt^* \quad (2)$$

In which $\psi\left(t - t^*\right) = \int_{-\infty}^{t} \frac{N(\tau)}{\tau} e^{\left[(t-t^*)/\tau\right]} d\tau$

$N(\tau)$ is the distribution function of relaxation times τ. In the above equation p_{ik} is the stress tensor, p is an arbitrary isotropic pressure, g_{ik} is the metric tensor of a fixed coordinate system x_i, and $e_{ik}{}^{(1)}$ is the rate of strain

tensor. It was shown by Walters [30] that equation (2) can be put in the following generalized form which is valid for all types of motion and stress

$$p^{*ik}\left(x,t\right)=2\int_{-\infty}^{t}\psi\left(t-t'\right)\frac{\partial x^{i}}{\partial x^{*m}}\frac{\partial x^{k}}{\partial x^{*\tau}}e^{[1]m\tau}\left(x,t\right)dt^{*} \quad (3a)$$

where x^{*i} is the position at times t^{*} of the element which is instantaneously at the point x^{i} at the time t. The fluid with equation of the state (1) to (3a) has been designated as the liquid B'. In the case of the liquid with short memories i.e. short relaxation times, the above equation can be written in the following simplified form:

$$p^{*ik}\left(x,t\right)=2\eta_{0}e^{[1]i\,k}-2k_{0}\frac{\partial e^{(1)i\,k}}{\partial t} \quad (3b)$$

In which $\eta_{0}=\int_{0}^{\infty}N\left(\tau\right)d\tau$ is limiting viscosity at the small rates of shear,

$k_{0}=\int_{0}^{\infty}\tau N\left(\tau\right)d\tau$ and $\frac{\partial}{\partial t}$ denotes the convicted time derivative.

The equation of conservation of electric charge is $\Delta.\vec{j}=0$ which gives $j_{y}^{*}=$ constant, where $\vec{j}=\left(j_{x}^{*},j_{y}^{*},j_{z}^{*},\right)$. Since the plate is electrically non-conducting, $j_{y}^{*}=0$ and is zero everywhere in the flow. Considering the magnetic field strength to be very large the generalized Ohm's laws including Hall current, in the absence of electric field neglecting the ion-slip and thermo electric effect takes the following form

$$\vec{j}+\frac{\omega_{e}\tau_{e}}{B_{0}}\left(\vec{j}\times\vec{B}\right)=\sigma\left(\vec{V}\times\vec{B}\right) \quad (4)$$

Where $\vec{V}$ is the velocity vector, ω_{e} is the electron frequency, σ is electrical-conductivity and τ_{e} is the electron collision time and

$$j_{x}^{*}=\frac{\sigma B_{0}}{1+m^{2}}\left(mu^{*}-w^{*}\right)$$

$$j_{z}^{*}=\frac{\sigma B_{0}}{1+m^{2}}\left(mu^{*}-w^{*}\right)$$

where $m=\omega_{e}\tau_{e}$ is the hall current parameter

Since the plate is infinite in extent all physical quantities are the function of y^{*} and t^{*} only. Thus the governing equations of flow under the usual Boussinesq approximation are:

$$\frac{\partial v^{*}}{\partial y^{*}}\Rightarrow v^{*}=-V_{0} \quad (5)$$

$$\frac{\partial u^{*}}{\partial t^{*}}+v^{*}\frac{\partial u^{*}}{\partial y^{*}}=\nu\frac{\partial^{2}u^{*}}{\partial y^{*2}}-k_{0}\frac{\partial^{3}u^{*}}{\partial y^{*2}\partial t^{*}}-\frac{\sigma\mu_{e}^{2}H_{0}^{\,2}}{\rho}\left(u^{*}-mw^{*}\right)+g\beta\left(T^{*}-T_{\infty}^{*}\right)-\frac{\nu}{k^{*}}u^{*} \quad (6)$$

$$\frac{\partial w^{*}}{\partial t^{*}}+v^{*}\frac{\partial w^{*}}{\partial y^{*}}=\nu\frac{\partial^{2}w^{*}}{\partial y^{*2}}-k_{0}\frac{\partial^{3}w^{*}}{\partial t^{*}\partial y^{*2}}-\frac{\sigma\mu_{e}^{2}H_{0}^{\,2}}{\rho}\left(w^{*}-mu^{*}\right)-\frac{\nu}{k^{*}}w^{*} \quad (7)$$

$$\frac{\partial T^*}{\partial t^*}+v^*\frac{\partial T^*}{\partial y^*}=\frac{k}{\rho C_p}\frac{\partial^2 T^*}{\partial y^{*2}}-\frac{Q_0}{\rho C_p}\left(T^*-T_\infty^*\right) \tag{8}$$

The boundary conditions are

$$\begin{aligned} &u^*=L^*\left(\frac{\partial w^*}{\partial y^*}\right), w^*=L^*\left(\frac{\partial w^*}{\partial y^*}\right), T^*=T^*{}_\infty+\left(T^*{}_w-T^*{}_\infty\right)e^{i\omega^* t^*} \quad at\ \ y^*=0 \\ &u^*\to 0, w^*\to 0, T^*\to T^*{}_\infty \qquad\qquad at\ \ y^*\to\infty \end{aligned} \tag{9}$$

Now we introduce the following non–dimensional parameters as follows:

$$\begin{aligned} &\eta=\frac{v_0}{\nu}y^*,\ t==\frac{v_0{}^2 t^*}{4\nu},\ \ u=\frac{u^*}{v_0},\ \ \ w=\frac{w^*}{v_0},\ \ \theta=\frac{T^*-T^*{}_\infty}{T^*{}_w-T^*{}_\infty} \\ &Gr=\frac{\nu\beta g\left(T^*{}_w-T^*{}_\infty\right)}{v_0{}^3},\qquad \Pr=\frac{\nu\rho C_p}{k},\qquad \phi=\frac{\nu Q_0}{\rho C_p v_0{}^2} \\ &\omega=\frac{4\nu w^*}{v_0{}^2}, h=\frac{v_0 L^*}{\nu}, M=\frac{\sigma B_0{}^2\nu}{\rho v_0{}^2}, K=\frac{k^* v_0{}^2}{\nu^2},\ \ \alpha=\frac{k_0 v_0{}^2}{4\nu^2} \end{aligned} \tag{10}$$

where θ the dimensional less temperature is, Gr is the Grashoff number, M is the Hartmann number, $\Pr$ is the Prandtl number, α is the visco-elastic parameter, ω is the frequency of the oscillations, h is the slip parameter. $T_\infty{}'$ denotes the temperature of the fluid far away from the plate, $T_w{}'$ denotes the temperature of the fluid at the plate, K is the thermal conductivity, C_p is the specific heat at constant pressure, ρ is the density of the fluid, β is the volumetric coefficient of thermal expansion, g is the acceleration due to gravity, ν is the molecular diffusivity, ϕ is heat generation and L^* is the characteristics length of the plate.

Equations (6) to (8) reduce to

$$\frac{1}{4}\frac{\partial u}{\partial t}+v^*\frac{\partial u}{\partial \eta}=\frac{\partial^2 u}{\partial \eta^2}-\alpha\frac{\partial^3 u}{\partial \eta^2\partial t}-\frac{M}{1+m^2}\left(mw+u\right)+Gr\theta-\frac{u}{K} \tag{11}$$

$$\frac{\partial w^*}{\partial t^*}+v^*\frac{\partial w^*}{\partial y^*}=\nu\frac{\partial^2 w^*}{\partial y^{*2}}-k_0\frac{\partial^3 w^*}{\partial t^*\partial y^{*2}}-\frac{\sigma\mu_e^2 H_0{}^2}{\rho}\left(w^*-mu^*\right)-\frac{\nu}{k^*}w^* \tag{12}$$

$$\frac{\partial T^*}{\partial t^*}+v^*\frac{\partial T^*}{\partial y^*}=\frac{k}{\rho C_p}\frac{\partial^2 T^*}{\partial y^{*2}}-\frac{Q_0}{\rho C_p}\left(T^*-T_\infty^*\right) \tag{13}$$

The corresponding boundary conditions become

$$\begin{aligned} &u=h\left(\frac{\partial u}{\partial \eta}\right), w=h\left(\frac{\partial w}{\partial \eta}\right), \theta=e^{i\omega t} \quad at\ \ \eta=0 \\ &u\to 0, \qquad w\to 0, \qquad \theta\to 0 \quad at\ \ \eta\to\infty \end{aligned} \tag{14}$$

Method of Solution

Introducing $q = u(\eta,t) + iw(\eta,t)$ and $i = \sqrt{-1}$, the equations (11) and (12) transform to

$$\frac{1}{4}\frac{\partial q}{\partial t} - \frac{\partial q}{\partial \eta} = \frac{\partial^2 q}{\partial \eta^2} - \alpha \frac{\partial^3 q}{\partial t\, \partial \eta^2} - \frac{M}{1+m^2}(1-im)q - \frac{q}{K} + Gr\theta \tag{15}$$

The corresponding boundary conditions become

$$\begin{aligned} &q = h\left(\frac{\partial q}{\partial \eta}\right), \theta = e^{i\omega t} \quad at \;\; \eta = 0 \\ &q \to 0, \qquad \theta \to 0 \quad at \;\; \eta \to \infty \end{aligned} \tag{16}$$

In order to solve the equations (13) and (15) under the boundary conditions (16), we assume

$$q(\eta,t) = q_0(\eta)e^{i\omega t} \quad and \quad \theta(\eta,t) = \theta_0(\eta)e^{i\omega t} \tag{17}$$

Substituting (17) into equations (13), (15) and (16), we obtain

$$(1-iA)q_0''(\eta) + q_0'(\eta) - [a_1 + i\,a_2]\,q_0(\eta) = -Gr\,\theta_0(\eta) \tag{18}$$

$$\theta_0''(\eta) + \Pr\,\theta_0'(\eta) - \left(\frac{i\omega \Pr}{4} + \phi \Pr\right)\theta_0(\eta) \tag{19}$$

The corresponding boundary condition reduce to

$$\begin{aligned} &q_0 = h\left(\frac{\partial q_0}{\partial \eta}\right), \theta_0 = 1 \quad at \;\; \eta = 0 \\ &q_0 \to 0, \qquad \theta_0 \to 0 \quad at \;\; \eta \to \infty \end{aligned} \tag{20}$$

Solving equations (18) and (19) under the boundary conditions (20) and using (17), we have

$$q(\eta,t) = \left[(a_{15} + i\,a_{16})e^{(a_3 + i\,a_4)\eta} - (a_1 + i\,a_2)e^{(a_5 + i\,a_6)\eta}\right]e^{i\omega t} \tag{21}$$

$$\theta(\eta,t) = \left[\Pr + \sqrt{\Pr^2 + (\phi \Pr + i\omega \Pr)}\right]e^{i\omega t - \frac{\eta}{2}} \tag{22}$$

Since $q = u(\eta,t) + iw(\eta,t)$, therefore from equation (21) we get

$$\begin{aligned} u(\eta,t) = {} & e^{-a_3\eta}\left\{a_{15}\cos(\omega t - a_4\eta) + a_{16}\sin(\omega t - a_4\eta)\right\} \\ & - e^{-a_5\eta}\left\{a_9\cos(\omega t - a_6\eta) + a_{10}\sin(\omega t - a_6\eta)\right\} \end{aligned} \tag{23}$$

$$\begin{aligned} w(\eta,t) = {} & e^{-a_3\eta}\left\{a_{15}\sin(\omega t - a_4\eta) + a_{16}\cos(\omega t - a_4\eta)\right\} \\ & - e^{-a_5\eta}\left\{a_9\sin(\omega t - a_6\eta) + a_{10}\cos(\omega t - a_6\eta)\right\} \end{aligned} \tag{24}$$

Separating (22) into real and imaginary parts, the real part is given by

$$\theta_r(\eta,t) = \cos\left[\omega t - \frac{\eta}{2}R_1 \sin\frac{\beta_1}{2}\right]e^{\frac{\eta\left(\Pr + R_1\cos\frac{\beta_1}{2}\right)}{2}} \tag{25}$$

Skin-friction

The axial component of the skin friction at plate for primary velocity is:

$$\tau_1 = \left(\frac{\partial u}{\partial \eta}\right)_{\eta=0} = \left[\left(a_9 a_5 - a_3 a_{15} - a_4 a_{16} + a_6 a_{10}\right)\cos \omega t + \left(a_{10} a_5 - a_3 a_{16} + a_4 a_{15} - a_6 a_9\right)\sin \omega t\right] \quad (26)$$

The transverse component of the shearing stress at plate for secondary velocity is:

$$\tau_2 = \left(\frac{\partial w}{\partial \eta}\right)_{\eta=0} = \left[\left(a_9 a_5 - a_3 a_{15} - a_4 a_{16} + a_6 a_{10}\right)\sin \omega t + \left(a_3 a_{16} - a_5 a_{10} + a_4 a_{15} + a_6 a_9\right)\cos \omega t\right] \quad (27)$$

RESULTS AND DISCUSSIONS

To demonstrate the effect of different parameters on the velocity and stress profiles in the previous segment, temperature and axial stress were included in the computational solutions for which the numerical model was developed, thus producing velocity and stress profiles that exhibit a particular set of axial and transverse stresses. In the case of the Prandtl series, the increase is set at 3.0, 5.0, and 10. This value of Prandtl 3.0 is equivalent to the one found in Freon. For example, there are several types of CFCs which include Freon in trade and commerce and businesses, and industries that use many CFCs. The exponency of the Prandtl value of 10, denotes a one standard atmosphere of gasoline. tensions have both grown over time In a capricious manner, the values of other parameters are selected. When w varies according to Grashoff number m, Grashoff number u and Prandtl number m, show the variables in (1) to (d) and (1) to (2) for) Grashoff and Hartmann number. These two results indicate a slowdown in primary velocity with an increase in Grashoff number, and an increase in Hartman number, but an increased current in the secondary velocity with the moment current for the expansion design generated by Grashoff, Hall, and a slower primary velocity for the design with the Prandtl phenomenon. Figures 1 (e) to (f) and 2 (e) demonstrate the influence of the visco-elasticity parameter on the main and secondary velocities. (figs. 1: uniform 2: comparing two different values). The stress distribution may be reduced by making Figure 1(e) clearly illustrates that secondary flow is amplified when the viscosity parameter is increased (Moving away from the plate produces stress in other places.) Additional decrease in slip velocity was observed at the original slip to improve flow while preserving primary velocity was revealed in these estimates. The secondary velocity is seen to decrease with the rise in the viscous and slip variables are made more obvious in Figure 2 (e). as colours that which change for Figures 1 (h) with C and and the colour changes in Figures 2 (g) are plotted according to K and the above pictures can be seen in the diagrams shown (h). It is observed from these examples that the primary velocity drops with the rise in porous medium permeability K, but the secondary velocity increases with the rising frequency parameter X. In the figure, figures 3 (A) to (C), you will see that as the Prandtl number increases, the number of oscillations decreases, and heat production slows down. On the rise in frequency, however, a change in temperature is detected. Also, on the curve, it is discovered that the high temperatures occur approximately at and then begin to decrease. Tables (or on page 2) show how the axial stress and transverse stress vary depending on the Grashoff number, Hall parameter, and Hartmann number. It can be concluded from these measurements that increasing Prandtl number ultimately induces axial stress and subsequently decreases it. In the other hand, something else, the other dimensions are expanding. Table (2) illustrates the inverse relationship between Prandtl number and Hartmann tension, but, Grashoff and Hall Current seem to raise stress. Relaxation of the viscoelastic and translatory tension are shown in table (3) and K for viscoelastic and K for visicoelastic and media respectively. The viscoelastic stress increases with anisotropic parameter, however the slip parameter and anisotropic stress decrease with the viselporous parameter. The permeability of the porous media, resulting in a negative shear stress, interferes and expands the overall stress on the portion

CONCLUSIONS

The findings of the analysis lead to the following prediction: As the Hall current parameter is raised, the secondary velocity reduces, but the Grashoff numbers rise. An interesting fact to consider is that in relation to Non-Newtonian fluids is that the primary velocity rises while remaining constant when you move away from the plate, however the secondary velocity declines when you move to another place (3)The primary velocity initially rises, and then decreases when (but is at a lower velocity) as it moves from the slip regime into the flow regime. In relation to flow in slip-flow, the secondary velocity profiles decrease. The temperature rises steadily over the first few degrees and quickly gets lower, and by a steep slope afterwards. As opposed to the Non-Newtonian fluids, the axial tension is elevated for the Newtonian fluids. As no-slip velocity is applied, the axial stress is lower but the transverse stress is higher.

REFERENCES

1. M S Alam, M M Rahman and M A Sattar (2006): MHD Free convection heat and mass transfer flow past an inclined surface with heat generation, *Thamasat. Int. J. Sci. Tech.* Vol. 11 (4), pp. 1 – 8.
2. D Chenna Kesavaiah, and B Devika (2020): Free convection and heat transfer of a Couette flow an infinite porous plate in the presence radiation effect, *Journal of Xi'an University of Architecture & Technology,* Vol. XII, (V), pp. 525-543
3. A J Chamkha (2004): Unsteady MHD convective heat and mass transfer past a semi- infinite vertical permeable moving plate with heat absorption, *Int. J. Eng. Sci.* 24, pp. 217 – 230.
4. D Chenna Kesavaiah and B Venkateswarlu (2020): Chemical reaction and radiation absorption effects on convective flows past a porous vertical wavy channel with travelling thermal waves, *International Journal of Fluid Mechanics Research,* Vol. 47 (2), pp. 153-169
5. R C Chaudhary and A K Jha (2008): Heat and mass transfer in elastic-viscous fluid past an impulsively started infinite vertical plate with Hall effect, *Latin American Applied Research,* 38, pp. 17-26.
6. C Derek, D C Tretheway and C D Meinhart (2002): Apparent fluid slip at hydrophobic micro channels walls, *Physics of Fluid,* 14, pp. L9 - L12.
7. S Karunakar Reddy, D Chenna Kesavaiah and M N Raja Shekar (2013): MHD heat and mass transfer flow of a viscoelastic fluid past an impulsively started infinite vertical plate with chemical reaction, *International Journal of Innovative Research in Science, Engineering and Technology,* Vol. 2 (4), pp.973- 981
8. F M Hady, R A Mohamed and A Mahdy (2006): MHD Free convection flow along a vertical wavy surface with heat generation or absorption effect, *Int. Comm. Heat Mass Transfer,* 33, 1253 – 1263.
9. M A Hossain, M M Molla and L S Yaa (2004): Natural convection flow along a vertical wavy surface temperature in the presence of heat generation/absorption, *Int. J. Thermal Science,* 43, pp. 157 – 163.
10. F N Ibrahim, A H Essawy, M R Hedar and T O Gad (2004): Flow of a visco-elastic fluid between coaxial rotating porous disks with uniform suction or injection, *Bulletin of Calcutta Mathematical Society*, 96(3), pp. 241-252.
11. N C Jain and P Gupta P (2007): Unsteady magneto polar free convection flow in slip flow regime with variable permeability and constant heat flux. *Journal of Energy, Heat and Mass Transfer,* 29, pp. 227-240.
12. A R A Khaled and K Vafai (2004): The effect of the slip condition on Stokes and Couette flows due to an oscillatory wall: exact solutions, *International Journal of Nonlinear Mechanics,* 39, pp. 795-809.
13. O D Makinde and E Osalusi (2006): MHD steady flow in a channel with slip at the permeable boundaries, *Romanian Journal of Physics*, 51, pp. 319-328.
14. M A Mansour, R A Mohamed, M M Abd-Elaziz and S E Ahmed (2007): Fluctuating thermal and mass diffusion on unsteady MHD convection of a micro polar fluid through a porous medium past a vertical plate in slip-flow regime, *International Journal of Applied Mathematics and Mechanics*, 3, pp. 99-117.
15. W Marques Jr, G M Kremer and F M Shapiro (2000): Couette flow with slip and jump boundary conditions, Continuum, *Mechanics and Thermodynamics,*12, pp. 379-386.

16. A Mehmood and A Ali (2007): The effect of slip conditions on unsteady MHD oscillatory flow of a viscous fluid in a planer channel, *Romanian Journal of Physics,* 52, pp.85-91.
17. S G Mohiddin, S V K Prasad and O A Beg (2010): Numerical study of unsteady free convective heat and mass transfer in a Walters-B Viscoelastic flow along a vertical cone, *International Journal of Mathematics and Mechanics.* 6, pp 88-114.
18. Srinathuni Lavanya, B Devika and D Chenna Kesavaiah (2020): Radiation effect on unsteady free convective MHD flow of a viscoelastic fluid past a tilted porous plate with heat source, *Journal of Xidian University*, Vol. 14 (4), pp. 2298 – 2312
19. T G Prasuna, M V R Murthy, N C P Ramachryulu and G V Rao (2010): Unsteady flow of a Viscoelastic fluid through a porous media between two impermeable parallel plates, *Journal of Emering Trends in Engineering and Applied Sciences,* 1(2), pp. 220-224.
20. M M Rahman and M S A Sarkar (2004): Unsteady MHD flow of viscoelastic Oldroyd fluid under time varying body force through a rectangular channel, *Bulletin of Calcutta Mathematical Society*, 96(6), pp. 463-470.
21. K R Rajagopal (1983): On Stokes problem for a non-Newtonian fluid, *Acta Mechanica,* 48, pp. 233-239.
22. Rajagopal K, Veena P H and Pravin V K (2006): Oscillatory motion of an electrically conducting visco-elastic fluid over a stretching sheet in saturated porous medium with suction/blowing, *Mathematical problem in Engineering,* (2006) pp. 1-14.
23. B Mallikarjuna Reddy, D Chenna Kesavaiah and G V Ramana Reddy (2019): Radiation and diffusion thermo effects of viscoelastic fluid past a porous surface in the presence of magnetic field and chemical reaction with heat source, *Asian Journal of Applied Sciences*, Vol. 7 (5), pp. 597-607
24. P K Sharma (2005): Fluctuating thermal and mass diffusion on unsteady free convection flow past a vertical plate in slip-flow regime, *Latin American Applied Research,* 35, pp. 313-319.
25. A K Singh and N P Singh (1996): MHD flow of a dusty viscoelastic liquid through a porous medium between two inclined parallel plates, *Proceeding of national Academy of Science India*, 66A (11) pp. 143-150.
26. K D Singh and R Kumar (2010): An exact solution of an oscillatory MHD flow through a porous medium bounded by rotating porous channel in the presence of Hall current, I*nternational Journal of Applied Mathematics and mechanics,* 6(13), pp. 28-40.
27. F Soltani and U Yilmazer (1998): Slip velocity and slip layer thickness in flow of concentrated suspensions, *Journal of Applied Polymer Science,* 70, pp 515-522.
28. D Chenna Kesavaiah, Srinathuni Lavanya and D Chandraprakash (2020): Chemical reaction effects on MHD flow over vertical surface through porous medium, *Gedrag & Organisatie Review*, Vol. 33 (2), pp. 134-151
29. M Bhavana and D Chenna Kesavaiah (2018): Perturbation solution for thermal diffusion and chemical reaction effects on MHD flow in vertical surface with heat generation, *International Journal of Future Revolution in Computer Science & Communication Engineering,* Vol. 4 (1), pp. 215-220
30. K Walters (1964): On second order effect in elasticity, plasticity and fluid dynamics, *IUTAM Int. Symp. (Reiner, M and Abir, D.eds.) Pergamon Press, New York.*

APPENDIX

$$a_1 = \frac{M}{1+m^2} + \frac{1}{K},\ a_2 = \frac{\omega}{2} - \frac{mM}{1+m^2},\ a_3 = \frac{1+R_2\cos\left(\frac{\beta_2}{2}\right) - R_2 A\sin\left(\frac{\beta_2}{2}\right)}{2\left(1+A^2\right)}$$

$$a_4 = \frac{A+R_2 A\cos\left(\frac{\beta_2}{2}\right) + R_2 A\sin\left(\frac{\beta_2}{2}\right)}{2\left(1+A^2\right)},\ a_5 = \frac{1}{2}\left[\mathrm{Pr}+R_1\cos\left(\frac{\beta_2}{2}\right)\right]\ a_6 = \frac{1}{2}\left[R_1\sin\left(\frac{\beta_1}{2}\right)\right]$$

$$a_7 = a_5^{\,2} - a_6^{\,2} + 2Aa_5a_6 - a_1 - a_5,\ a_8 = 2a_5a_6 - a_2 - a_6 + Aa_5^{\,2} + Aa_6^{\,2},\ a_9 = \left(\frac{a_7}{a_7^{\,2} + a_8^{\,2}}\right)$$

$$a_{10} = Gr\left(\frac{a_8}{a_7^{\,2} + a_8^{\,2}}\right),\ a_{11} = a_5a_9 + a_6a_{10},\ a_{12} = a_6a_9 - a_5a_{10},\ a_{13} = a_9 + ha_{11}\ a_{14} = a_{10} - ha_{12},$$

$$a_{15} = \frac{a_{13} + h\left(a_3a_{13} - a_4a_{14}\right)}{\left(1+ha_3\right)^2 + \left(ha_4\right)^2},\ a_{16} = \frac{a_{14} + h\left(a_4a_{13} + a_3a_{14}\right)}{\left(1+ha_3\right)^2 + \left(ha_4\right)^2}\ \beta_1 = \tan^{-1}\left(\frac{\omega}{\Pr}\right), A = \alpha\omega,$$

$$\beta_2 = \tan^{-1}\left[\frac{4\left(a_2 - Aa_1\right)}{1+4\left(a_1 + Aa_2\right)}\right],\ R_1 = \Pr^{\frac{1}{2}}\left[\left(\Pr^2 + 4\phi\Pr\right) + \omega^2\right]$$

$$R_2 = \left\{\left[1+4\left(a_1 + Aa_2\right)\right]^2 + 16\left(a_2 - Aa_1\right)^2\right\}^{\frac{1}{4}}$$

Table (1): Axial shearing stress τ_1

Gr	Pr	m	M	τ_1
2	3	0.5	5	-0.35
4	3	0.5	5	-0.75
2	10	0.5	5	-0.01
2	2	1.0	5	-0.08
2	2	0.5	5	-0.08
2	2	0.5	10	-0.35

Table 2: Transverse shearing stress τ_2

Gr	Pr	m	M	τ_2
2	3	0.5	5	0.25
4	3	0.5	5	0.52
2	10	0.5	5	0.10
2	2	1.0	5	0.28
2	2	0.5	5	0.31
2	2	0.5	10	0.20

Table 3: Axial shearing stress τ_1

α	h	K	τ_1
0.05	0.2	1.0	-0.038
0	0.2	1.0	-0.038
0.2	0.2	1.0	-0.038
0.05	0	1.0	-0.038
0.05	0.4	1.0	-0.030
0.05	0.2	3.0	-0.042

Table 4: Transverse shearing stress τ_2

α	h	K	τ_2
0.05	0.2	1.0	0.26
0	0.2	1.0	0.26
0.2	0.2	1.0	0.26
0.05	0	1.0	0.42
0.05	0.4	1.0	0.18
0.05	0.2	3.0	0.28

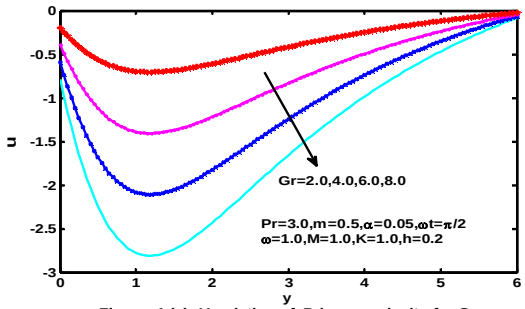

Figure 1 (a): Vaariation of Primary velocity for Gr

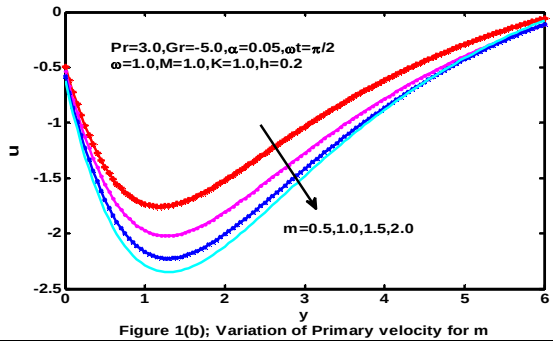

Figure 1(b); Variation of Primary velocity for m

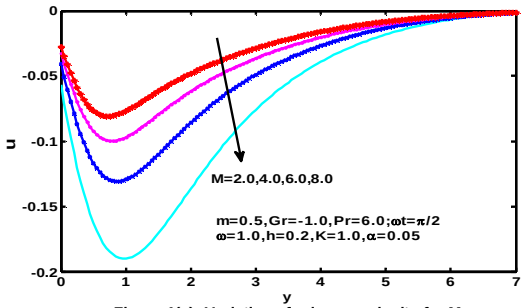

Figure 1(c): Variation of primary velocity for M

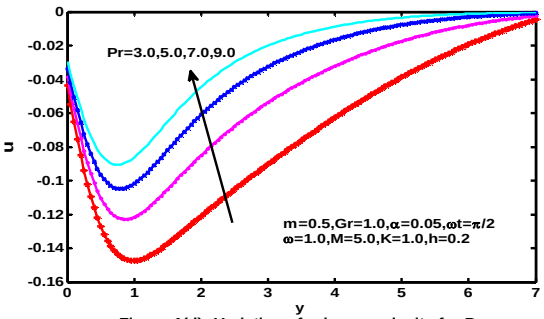

Figure 1(d): Variation of primary velocity for Pr

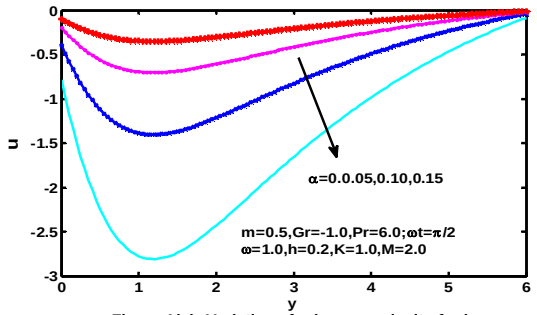

Figure 1(e): Variation of primarya velocity for h

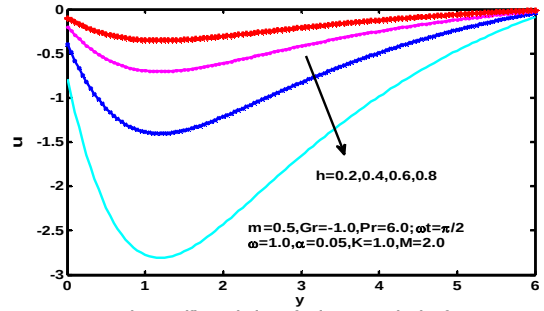

Figure 1(f): Variation of primarya velocity for α

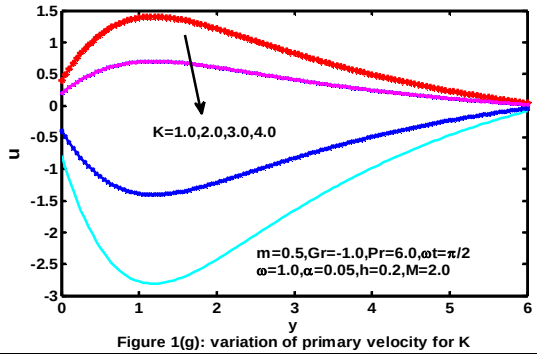

Figure 1(g): variation of primary velocity for K

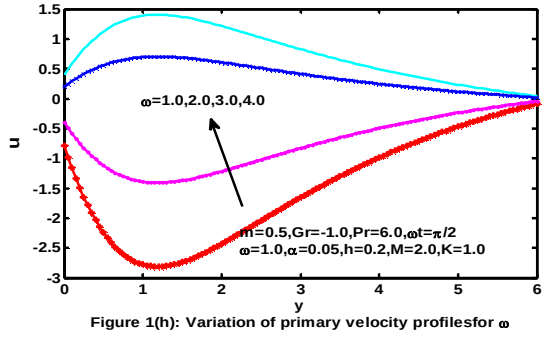

Figure 1(h): Variation of primary velocity profilesfor ω

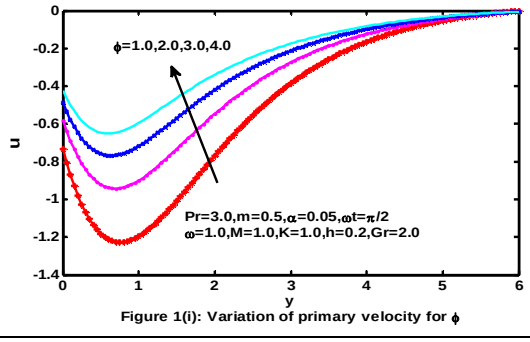

Figure 1(i): Variation of primary velocity for ϕ

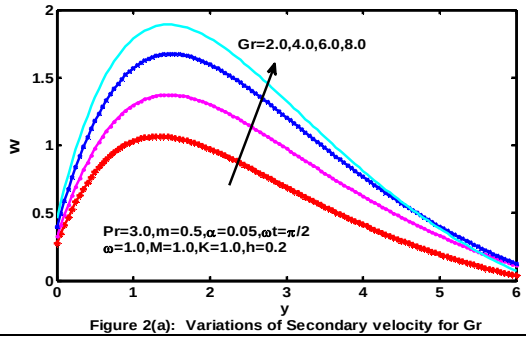

Figure 2(a): Variations of Secondary velocity for Gr

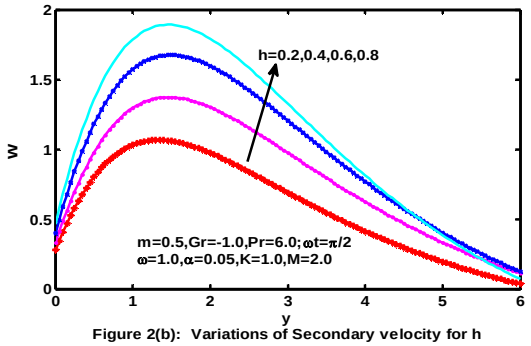

Figure 2(b): Variations of Secondary velocity for h

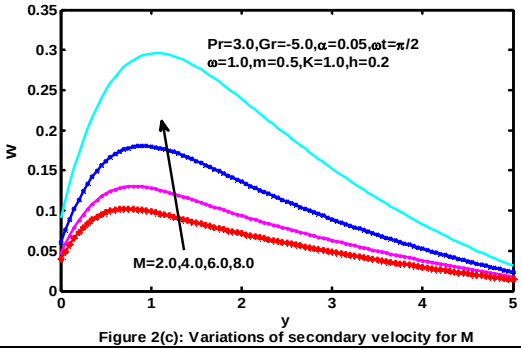

Figure 2(c): Variations of secondary velocity for M

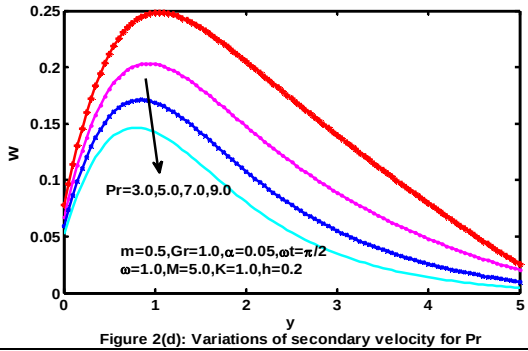

Figure 2(d): Variations of secondary velocity for Pr

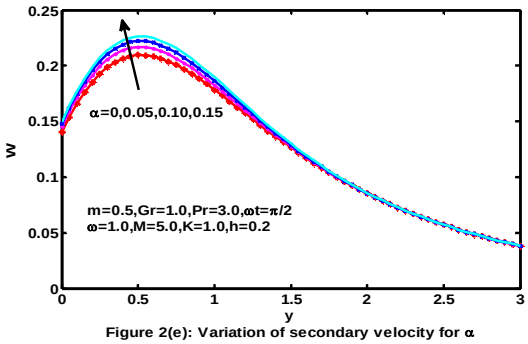

Figure 2(e): Variation of secondary velocity for α

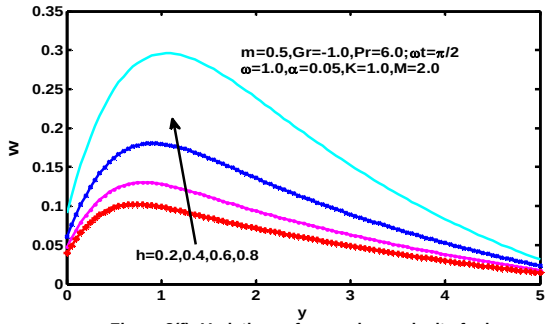

Figure 2(f): Variations of secondary velocity for h

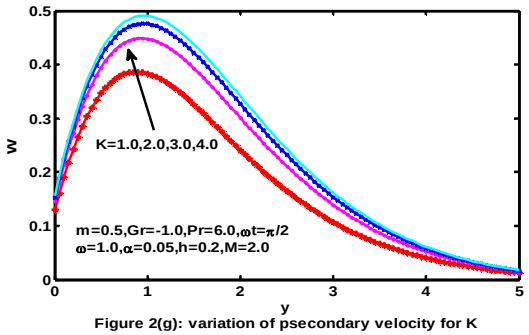

Figure 2(g): variation of psecondary velocity for K

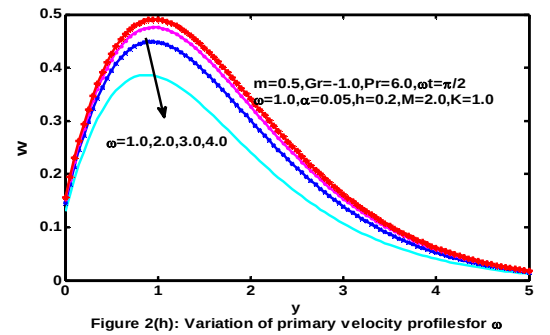

Figure 2(h): Variation of primary velocity profilesfor ω

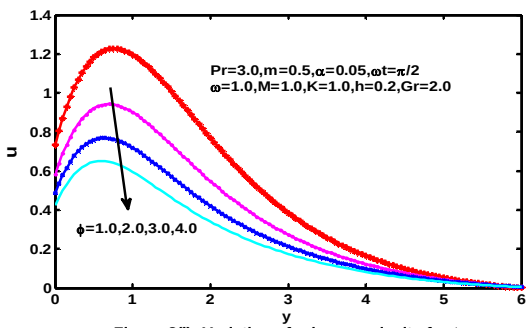

Figure 2(i): Variation of primary velocity for ϕ

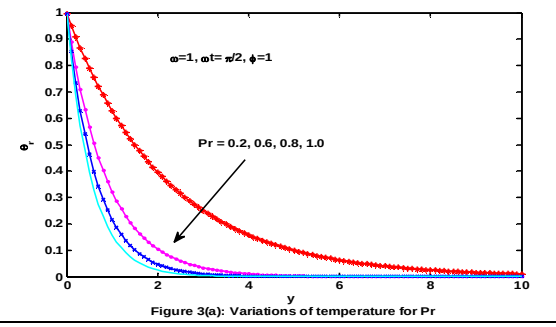

Figure 3(a): Variations of temperature for Pr

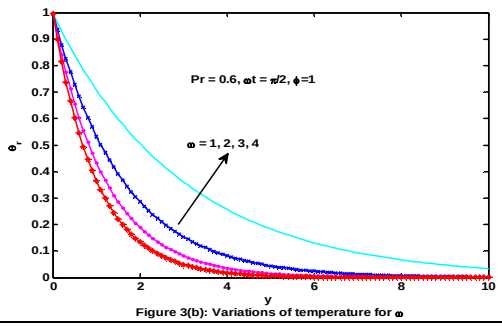

Figure 3(b): Variations of temperature for ω

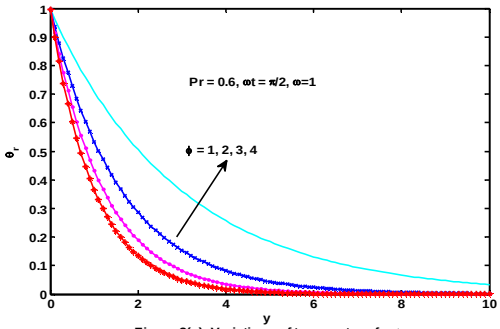

Figure 3(c): Variations of temperature for ϕ

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RESEARCH ARTICLE

Exotic Fish Tilapia (*Oreochromis mossambicus* Peters 1852) and its Impact on Ichthyofauna and Fish Production of Jaisamand Lake (India): A Statistical Approach

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ABSTRACT

Tilapia, (*Oreochromis mossambicus* Peters, 1852) is an exotic fish and well established in aquatic environment of lake Jaisamand, India. The present paper elucidated the impact of this exotic fish on faunal diversity and fish production of lake during the years 1988-2019. The result showed that in 1988-89 existence of tilapia was negligible but it was dominated in 2001-2002 and contributed about 98% of the fish production. The catch composition depicted that major carps (36.8%), minor carps (53.6%) and catfishes (9.5%) together contributed the production in 1990-91 but later on contribution of tilapia was increased and dominated by 98.0% whereas major carps was 0.6%, minor carps 0.4% and catfishes 1.0% in 2001-2002, in 2010-11 major carps 11.0%, minor carps 5.6%, catfishes 3.5% and tilapia was found dominated by 80.0%. in contrast in 2018-19 contribution of indigenous fauna was little high (major carps 29.4%, minor carps 25.0% and catfishes 28.3%) and tilapia declined to 17.2%. Similarly, decline in fish fauna diversity due to invasion of tilapia were also observed in studied water body. The statistical analysis including ANOVA, multiple comparison and Tukey HSD test showed significant difference between the indigenous fishes and tilapia which revealed that entry and survival of tilapia adversely impacted indigenous fauna including carps and catfish in Jaisamand Lake.

Keywords: Jaisamand Lake, tilapia, exotic fish, fish production, invasion.

INTRODUCTION

Rajasthan is the largest state of India constituting vast area of fishery resources in the form of reservoirs (1736), ponds and tanks (13682) that cover an area of 4.24 lakh ha while rivers and canals occupy 30,000 ha and water-logged area covers 80,000 ha [1]. Among these water resources, Jaisamand Lake is one of the largest manmade and

productive water body [2]. The morphometric features and aquatic ecological silhouettes of the reservoir supported the high biological production [3,4,5,6) and it was considered important water for the fish production of the state especially for Indian major carps [7,8]. In India, huge number of exotic fishes have been introduced during the last half of the century for different purposes including aquaculture, sport fishing, ornamental fish, mosquito control etc. [9,10] and these were well established in Indian waters [11,12,13] and resulted environmental and ecological disturbances for indigenous fauna and fish production [14]. The invasion of these exotic non-native fishes is serious threat to the native fish fauna and water bodies as studied by different researchers in this aspect [12,13,14,15,16,17,18]. Similarly, in Jaisamand Lake exotic fish tilapia (*Oreochromis mossambicus* P.) was first time observed during 1991[19,20] and it was reported that fish production and faunal diversity of the lake was adversely affected by the invasion of this exotic fish [3,5,12,21]. The present paper highlights the impacts of introduced exotic fish tilapia (*O. mossambicus*) on native biodiversity of indigenous fauna and overall fish production of Jaisamand Lake which were statistically verified.

MATERIALS AND METHODS

Study area

Jaisamand Lake is one of the largest man-made freshwater lake constructed in 1729 AD across the Gomati River about 56 km away in South East of Udaipur (Rajasthan), geographical location of 73° 57' E longitude and 24° 14' N latitude at altitude of 587 m (MSL) and water spread of the lake is 7,160 ha with maximum depth of 32 m and mean depth of 15 m.

Data Collection and analysis

The annual fish production data and information on the fish faunal diversity from 1988-89 to 2018-19 of Jaisamand Lake were collected from Department of Fisheries (Government of Rajasthan), Udaipur. These data reanalyzed to assess relative share, effect on fish production and impact on fish faunal diversity. These data were evaluated for descriptive statistics, ANOVA, multiple comparison of the fish groups to statistically justify and verify the described impact of exotic fish tilapia on indigenous fauna production of Jaisamand Lake. Statistical analysis and graphical presentation were prepared by SPSS 16.0 and MS excel 2010.

RESULT AND DISCUSSION

The total fish production of Jaisamand Lake was 306.00 MT in 2018-19 which comprises different categories e.g., indigenous fishes: Indian major carps, minor carps, catfishes and exotic fish species, tilapia (Fig. 1). Figure 1 showed that during 1988-89 fish production included only indigenous fishes and in the year 1991-1992 few specimens of tilapia was introduced that later on dominated the fish production of Jaisamand Lake up to 98.00%. In 1990-91, fish production was 287.150 mt that composed of Indian major carps 36.8%, minor carps 53.6% and catfishes 9.5%, in 2000-01 it was 369-978 mt that included Indian major carps 0.6%, minor carps 0.4%, catfishes 1.0% and tilapia 98.0% in 2010-11 it was 229.470 mt that included Indian major carps 11.0%, minor carps 5.6%, catfishes 3.5% and tilapia 80.0% whereas, in 2018-19 fish production was 306.00 mt that constitute Indian major carps 29.4%, minor carps 25.0%, catfishes 28.3% and tilapia 17.2% (Fig. 2). This reduction in Tilapia population might be due to control measures taken in Jaismad Lake as reported by Durga and Srivastava [22]. Similarly, Kumari [23] also reported that for controlling Tilapias in Jubilee Lake certain remedial measures such as selective fishing and even under sized Tilapia's removal were practiced with special permission from the state Fisheries Department. Similarly, for few years' catfish catching was stopped and Indian major carps fingerlings were stocked in adequate numbers. These controlling measures observed good recovery in the production of IMC and other indigenous fishes.

NBFGR [24] reported only 38 fish species in Jaisamand Lake (Rajasthan). The declining trend of fish species depicted that tilapia was dominated and dominated all other indigenous fish fauna viz. major carps, minor carps, catfishes

and so many other fishes in Lake Jaisamand. Sharma and Johal [7] reported 41 fish species in Jaisamand lake which was reduced to 40 as documented by Johal and Chahal [25].

The above results were established by multiple statistical analysis that showed that production of catfishes was consistent throughout the study period with less deviation (6.825) followed by the major carps (11.017), minor carps (11.543) and tilapia (26.587) since last three decades (Table 1). The ANOVA test for fish production data indicated significant difference at P <0.05 (Table 2). These data were further analyzed for Tukey HSD test to find out the significant difference in fish production on the basis of fish group and concluded that tilapia production had significant difference in the production with respect to other groups of fishes (P< 0.05) whereas other fish groups (major carp, minor carp and catfish) showed no significant difference in production (Table 3). Similarly mean production for different fish groups were displayed in homogenous subsets revealed that major carps, minor carps and catfishes showed similar production within same subset whereas, production of tilapia was significantly different with other fish groups falling in another subset (Table 4). The results of ANOVA, multiple comparison and Tukey HSD test showed insignificant difference among the production of major carp, minor carp and catfish which revealed that these fish groups survive in the water body without competition and fish production was not affected while entry and survival of tilapia in the Jaisamand Lake adversely impacted indigenous fauna including carps and catfish that may be attributed by omnivores feeding habit, competition for food, competition for space etc. of tilapia with other fishes. Although, report on statistical analysis and verification of the results on invasion of tilapia are not available but Sugunan [26] reported that Pambar reservoir in Tamil Nadu was the shelter for population of tilapia, since 1960s and contributing noticeably in commercial catches. The fluctuation in fish production and impact on total production of Jaisamand Lake were also reported by different researchers [3,8,19,22]. Similarly, Courtenary and Hensley and Bhagat and Dwivedi [27] reported decrease in population size of established fishes due to overcrowding of tilapia. Similarly declining trend in fish species of Jaisamand lake (Rajasthan) was observed in different studies [5,7,24,25].

REFERENCES

1. Jhajhria A. (2017). Conservation of Freshwater Fish Biodiversity: A Challenge for Rajasthan. International Journal of Scientific Research and Management, Vol. 5(06):5445-5447
2. Durve, V S (1976). The Fisheries of Lake Jaisamand, Rajasthan. J. Inland Fish. Soc. India, Vol. 8: 19-23
3. Ujjania, N C (1997). Some aspects of fishery and biology of exotic fish Tilapia (*Oreochromis mossambicus* P.) from Lake Jaisamand Udaipur (Rajasthan). M.Sc. Ag. (Lim. And Fish) thesis, Rajasthan Agricultural University, Bikaner.
4. Kohli, M.P.S., Prakash, C. and Jhadhav, R.J. (1998). Aquatic productivity indices in four tribal districts of Rajasthan with special reference to fisheries development. *Punjab Fisheries Bulletin* 20:53-62.
5. Balai, Vijay Kumar (2007). Current Fish and Planktonic Biodiversity in the Reservoir Jaisamand Udaipur, Rajasthan. Ph.D. thesis, College of Fisheries, Maharana Pratap University of Agriculture nad Technology, Udaipur
6. Ujjania, N.C., L.L.Sharma and V.P.Saini (2020). Congenial aquatic environment and invasion of exotic fish tilapia (*Oreochromis mossambicus* P. 1852) in Jaisamnd Lake, India. J. Ento. Zool. Std., Vol. 8(1):1670-1673
7. Sharma, K P and Johal, M S (1982). On the fish and fisheries of Jaisamand Lake Rajasthan, India. West. Cs. Spolec. Zool., Vol. 46: 56-59
8. Singh, S. (1994). Some aspects of fishery and biology of *Labeo rohita* (Ham.) from Lake Jaisamand, Rajasthan, M.Sc. (Ag.) Thesis, Raj. Agricultural University, Bikaner.
9. Bijukukar, A. (2000). Exotic fishes and freshwater diversity, Zoos. Print J., 15(11): 363-367
10. Raman, Ram Praksh, Mishra, Arvind, Sanjeevan Kumar, Soni Sahay, Manij N. Bhagat, and Saurav Kumar (2013). Introductions of exotic fish species into Indian waters: an overview of benefits, impacts, issues and management.

In; Advances in Fish Research, Eds: U.C. Goswami. Publisher: Narendra Publishing House, Delhi, India. pp. 257-271

11. Singh A K, Lakra W S (2011). Risk and benefit assessment of alien fish species of the aquaculture and aquarium trade into India. Rev. Aquac. Vol. 3: 3-18
12. Ujjania, N C, Sharma L L and Srivastva R M (2013). Assessment of age and growth of exotic fish tilapia (*Oreochromis mossambicus* P.) in lake Jaisamand, India. Indi. J. Funda. Applied Life Sci., Vol. 3 (4): 27-34
13. Mayank, P and Dwivedi, AC (2017) Resource Use Efficiency and Invasive Potential of Non-Native Fish Species, *Oreochromis niloticus* from the Paisuni River, India. Poult. Fish. Wildl. Sci., 5: 177
14. Canonico, G C, Arthington A, McCrary J K, Thieme M L (2005). The effects of introduced tilapias on native biodiversity. Aquat. Conserv. Mar. Freshw. Ecosyst. Vol. 15(5):463-483
15. Singh A K, Lakra W S (2006) Impact of alien fish species in India: emerging scenario. J. Ecophysiol. Occup. Health, Vol. 6 (3–4): 165–174
16. Lakra, WS, Singh, A.K. and Ayyappan S. (2008). Fish Introductions in India: Status, Potential and Challenges. Narendra Publishers, New Delhi, India
17. Arthur, Robert I, Kai Lorenzen, Phansy Homekingkeo, Kamchanh Sidavong, Bounthong, Sengvilaikham, Caroline J. Garaway (2010). Assessing impacts of introduced aquaculture species on native fish communities: Nile tilapia and major carps in SE Asian freshwaters. Aquaculture, Vol. (299): 81–88
18. Ahmad, Sultanul Arafin Shemeen, Amrit N Bart, Yang Yi, James E Rakocy and James S Diana (2010). The effect of the introduction of Nile tilapia (*Oreochromis niloticus*, L.) on small indigenous fish species (mola, *Amblypharyngodon mola*, Hamilton; chela, *Chela cachius*, Hamilton; punti, *Puntius sophore*, Hamilton). Aquaculture Research, Vol. (41): 904-912
19. Gupta, A K and Jain, A K (1994). Occurrence of tilapia in Lake Jaisamand: A threat to indigenous fishery resources of Rajasthan. Fishing Chimes, 1994: 15
20. Anon (1995). Menace of Indian fishes of Lake Jaisamand due to occurrence of African fish. Rajasthan Patrika, 11.12.1995
21. Ujjania, NC, Sharma, LL, Dubey, Monika and Srivastva, RM (2015). Bio Invasion of Exotic Fish Tilapia (*Oreochromis mossambicus* P. 1852) and its impact on indigenous Fish Fauna of Lake Jaisamand. *Int. J. Fish. Aqua. Std.*, 2(6): 174-177
22. Durga I A and Srivastava, R M (2008). Tilapia invasion and its control in Jaisamand Lake, Udaipur: A case study. *In*: Mangement of freshwater ecosystem, (*Eds*.) Sharma *et al,* Agrotech Publishing Academy, Udaipur. 327-335
23. Kumari S. (2018) The changing consequences of Tilapia invasion in the Jayanti Sarovar of Jamshedpur, India: Control implication. Biospectra ,13(1), 9-12
24. NBFGR, (2003). Annual Report 2002-03. National Bureau of Fish Genetic Resources (NBFGR), Lucknow, India
25. Johal, M.S. and Chahal, J.S. (1988). Importance of faunal studies of lakes in fisheries development. *Proceedings of National Symposium on Past, Present and Future of Bhopal Lakes,* 115-121
26. Sugunan, V V (1994). Exotic fishes and their role in the reservoir fisheries of India. J. Inand Fish. Soc. India, Vol. 26(1): 125-132
27. Courtenary, W R Jr and Hensley, D A (1979). Range expansion in southern Florida of introduced spotted tilapia, with comments on its environmental impress. *Environmental Conservation,* Vol.6 (2): 149-151

Table 1. Descriptive statistics of variables (different groups of fishes contributing total fish production)

Fish groups	Fish production (MT)			
	Minimum	Maximum	Mean	Standard Error
Major carp	1.29	218.57	63.569	11.017
Minor carp	0.89	239.70	63.544	11.543
Catfish	1.38	125.12	27.746	6.825
Tilapia	0.00	591.46	142.456	26.587

Table 2. ANOVA for significant relations between different groups of fishes

Fish group	Sum of Squares	df	Mean Square	F	Significance
Between Groups	218345.757	3	72781.919	9.316	0.000
Within Groups	937468.684	120	7812.239		
Total	1155814.441	123			

Table 3. Multiple comparisons for significant relations between different groups of studied fishes

Fish group (I)	Fish group (J)	Mean Difference (I-J)	Significance
Major carp	Minor carp	0.025	1.000
	Catfish	35.823	0.385
	Tilapia	-78.887*	0.003*
Minor carp	Catfish	35.798	0.386
Minor carp	Tilapia	-78.912*	0.003*
Catfish	Tilapia	-114.710*	0.000*

*The mean difference is significant at the 0.05 level

Table 4. Tukey HSD test of means for fish groups in homogenous subsets

Fish group	N	1	2
Major carp	31	63.569	
Minor carp	31	63.544	
Catfish	31	27.746	
Tilapia	31		142.456
Significance		0.385	1.000
			Subset for alpha = 0.05

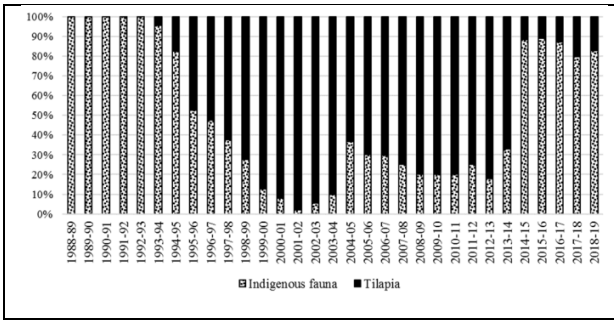

Figure 1. Total fish production including indigenous fauna and exotic fish tilapia in Jaisamand Lake

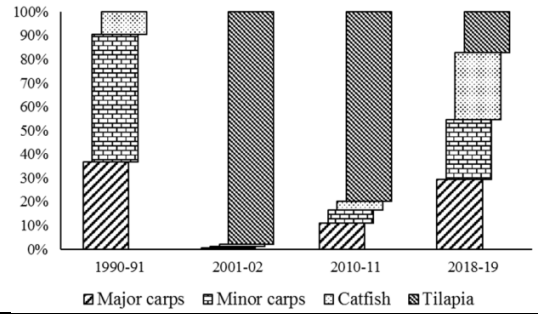

Figure 2. Contribution (%) of indigenous fauna (major carps, minor carps and catfishes) and exotic fish tilapia in total fish production of Jaisamand Lake

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RESEARCH ARTICLE

In silico Molecular Docking Study of Chicoric Acid from *Ocimum sanctum* (Tulsi) Working against *Achromobacter xylosoxidans* Causing Bronchitis

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ABSTRACT

Phytochemicals are present in every part of medicinal plants are maintain an essentiality for the benefits required in various living organisms. These bioactive chemical compounds are helpful against many causative organisms. Here in this *in silico* study it has been accounted that *Ocimum sanctum* (Tulsi) plant extract is utilized to fix Bronchitis. Bronchitis a condition in which cold and flu like symptom may appear in acute condition. If it not treats properly on time it may leads to severe lungs infection. Now-a-days this lungs infection might became sensitive to get affected by COVID virus. *Achromobacter xylosoxidans* can cause bronchial infection and sometimes it turned in to multidrug resistant. In our docking study, we discovered phytochemicals from tulsi plant are effective against the protein compound of gram-negative *A. xylosoxidans* bacteria. We likewise presumed that, *in silico* docking, the computational tools help to find out remedial treatment as a medication against chronic bronchitis using phytochemicals. The AutoDock Vina allows the user to upload a structure file as a pdb format for a protein and ligand and provide a result.

Keywords: Phytochemicals, AutoDock Vina, *Achromobacter xylosoxidans, Ocimum sanctum*

INTRODUCTION

In this Pandemic era the abnormal symptom or condition related to lungs infection i.e., cough or cold may reflects a severe consequence. People having symptom related to lungs infection are having high risk to getting infected by COVID. Bronchitis also a disease or condition in which people may often cough up thicken mucus, which can be

discoloured. This infection is associated with lining of bronchial tube which may lead to the lungs. This leads to a chronic condition which included in chronic obstructive pulmonary disease. If the Bronchitis condition will not be treated it can cause serious lungs injury which leads to pneumoniae. Mainly immunocompromised patients are having high risk to get infected by this opportunistic pathogen *Achromobacter xylosoxidans*. This is a gram negative motile aerobic bacterium which can cause various hospital acquired infections as well as community acquired infections; like cystic fibrosis, lungs infection, septic shock, Bronchitis etc in both animal and human. Recurrent deadly infections cause through several microbes and they became multi drug resistant. Due the capability of becoming resistant against various antibiotics, diseases are very difficult to diminish from their root [1].

Achromobacter xylosoxidans is an aerobic, non-fermenting Gram-negative bacillus that is capable of oxidizing xylose [9]. It is a gram-negative bacterium, has got its recognition [11] as an emerging nosocomial pathogen during the last decade which can cause infection in humans for e. g. endocarditis [12], bacteremia [13], ocular infections [14] or urinary tract infection [15]. It also plays a vital role in colonization, infecting the CF patients [16,17,18,19,20] and can cause pneumonia in elderly or immunocompromised non-CF patients [7]. Now-a-days scientists are performing research over phytochemicals extracted from different medicinal plants. Some phytochemicals were found very effective against the infection causing strain. *Ocimum tenuiflorum*, or Tulsi, or Holy Basil which belongs to the family Lamiaceae has an existence of origin in north central India. This aromatic shrub now grows throughout the tropical regions of Eastern World. It is believed that, this shrub has both spiritual and medicinal properties, and within Ayurveda, it is known as "The Queen of Herbs", "Mother Medicine of Nature". India has been adopting this shrub for its spiritual rituals and common life styles practices which includes the stability in the body well-being. The development in the field of science on tulsi, which supports old Ayurvedic intelligence, claims that tulsi has been an analeptic for the body, psyche and soul that offers cure to most advanced medical conditions [8].

Tulsi is maybe probably the best illustration of Ayurveda's comprehensive way of life way to deal with wellbeing. It is said that, tulsi has the capability to penetrate the deep body tissues, secretion of dry tissue and also normalize kapha and vata. It is also said that Tulsi should be consumed daily for the prevention of disease, for good well-being and longevity and in daily life, it also assists in dealing with stresses. Tulsi is likewise attributed with offering brilliance to the appearance, pleasantness to the voice and cultivating excellence, knowledge, endurance and a quiet enthusiastic attitude. Notwithstanding these wellbeing advancing properties, tulsi is suggested as a treatment for a scope of conditions including nervousness or anxieties, coughing, dysentery, asthma, fever, diarrhoea, joint inflammation, eye infections, otalgia, indigestion, hiccups, regurgitating or vomiting, gastric, heart and genitourinary problems, back torment, diseases especially of skin, ringworm, bugs, snake and scorpion chomps and intestinal sickness and malaria [8].

Considered as a powerful adaptogen, tulsi has an unique combination of pharmacological activities that advance prosperity and versatility. While the idea of an "adaptogen," or herb that assists with the transformation to stress and the advancement of homeostasis, isn't broadly utilized in Western medication, Western science has uncovered that tulsi does in fact have numerous pharmacological activities that satisfy this reason [8]. The therapeutic properties of tulsi have been concentrated in many scientific investigations which includes in-vitro, creature and human tests. These investigations uncover that tulsi has an interesting mixture of activities that include: Antimicrobial (counting antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, anthelmintic), antimalaria, against diarrheal, hostile to oxidant, hostile to oxidant, mitigating, chemo preventive, radioprotective, hepato-defensive, neuro-defensive, cardio-defensive, hostile to diabetic, against hypercholesterolemia, hostile to hypertensive, against cancer-causing, pain relieving, against pyretic, hostile to unfavourably susceptible, immunomodulatory, focal sensory system depressant, memory improvement, against asthmatic, against tussive, diaphoretic, hostile to thyroid, against fruitfulness, hostile to ulcer, hostile to emetic, against fitful, against joint, adaptogenic, against stress, against cataract, against leukodermal and against coagulant activities. These pharmacological activities help the body and brain adapt to a wide range of chemical, physical, irresistible and enthusiastic anxieties and re-establish physiological and mental capacity [8].

The studies on the standard virtual docking consists of ligands which are freely docked into a rigid receptor. There is a wide acceptance in ligand-protein complexes, that the flexibility of side chain has a major vital role. According to this orientation of the ligand, the receptor alters its binding site position, which is made possible by the changes in the ligand-protein complexes [2]. Amorim et al., resulted through the research held on 2015 that total from 43 numbers of isolated organism, 5% of bacteria causing respiratory infection is *A. xylosoxidans*. [3]. CF patients, *A. xylosoxidans* causes inflammation and clinical deterioration which is similar to that of P. aeruginosa and clinically, it is one of the important pathogens.

There is a history of biofilm in sputum in those CF patients with chronic mucoid P. Aeruginosa lung infection. [8]. The biofilms of *A. xylosoxidans* and Bcc is comparatively similar to that of P. aeruginosa biofilms in sputum with a dark, condensed appearance where the bacteria are closely packed in contrast to the biofilm of mucoid *P. Aruginosa* where the abundant light alginate matrix, where the bacterial cells will lead to its separation [4]. Marsac et al., concluded in the study conducted on 2021 that, Chronic lung disease is generally associated with *A. xylosoxidans* lung infection, severe in children with CF. This pathogen was considered to be an infrequent bacterium which infects the respiratory tract of CF patients. Bigger imminent investigations surveying the pathogenicity of this arising microbe just as global treatment proposals are desperately justified [5].

MATERIALS AND METHODS

AutoDock Vina is a docking software tool which is used for targeting drug against disease causing microorganism. Many researches proven that the medicinal plants contain phytocompounds like alkaloids, essential oils, phenols, flavonoids and ethanol and water-soluble compounds. Therefore, those essential plant derived compounds mostly very much useful for drug design in pharmaceutical company due to its ethnomedicinal activity & proven utility as traditional drugs to cure many diseases (Mbatchou et al., 2011). Before a decade, Ocimum sanctum (Tulsi) has been utilized as a traditional remedy for many infections. It has plentiful with bio active chemical compounds such as, Chicoric acid acid, Eugenol, Carvacrol, Linalool, and Beta Caryophyllene. These phytocompounds have been extensively worked to cure many infections as well as used in food products. It has accounted for that Ocimum sanctum plant is a holy plant and its extract contains several antibacterial activity and work as a remedy against many diseases. It has carried several bio active compounds which are very effective against diseases and disease-causing bacteria. Here in this study, we checked the activity of Protein Glyceraldehyde 3-phosphate Dehydrogenase present in *A. xylosoxidans* and interaction between Chicoric acid acid extracted from Ocimum sanctum plant. Here we use RCSB protein data bank for checking structure of different enzymes produced by the organism as well as PubChem data base for ligand structure for perform molecular docking.

RCSB This asset is powered by the Protein Data Bank file data about the 3D shapes of proteins, nucleic acids, and complex gatherings that helps students and analysts see all parts of biomedicine and agribusiness, from synthesis of protein to wellbeing and sickness or disease. Freely accessible chemical information is largely collected by PubChem. Chemicals can be searched by using the name, formula of that chemical, its structure, or any other identification. Both chemical and physical properties, the biological activities, its safety and the level of toxicity, patents, the citation of literature and much more. AutoDock Vina gives most accurate result for binding interaction of targeted ligand and protein to discover drug.

RESULT AND DISCUSSION

AutoDock Vina molecular docking study, helps to found good ΔG Kcal/mol value with lupeol. The ΔG Kcal/mol value was about -8.2. This is a very reliable software which helps to get ΔG Kcal/mol was obtained in terms of full fitness. The value with a smaller number of ΔG shows, the successful approach of phytochemical Chicoric acid acids from *Ocimum sanctum* (Tulsi) as a drug against emerging infectious pathogen *A. xylosoxidans* producing

Glyceraldehyde 3-phosphate Dehydrogenase enzyme. Chicoric acid acids can be a potent phytochemical for Cystic fibrosis.

CONCLUSION

In this present study about remedy of Bronchial infection, found some ethnomedicinal activity of *Ocimum sanctum* (Tulsi). The leave as well as flower extract of this medicinal plant, traditionally utilized as a remedy of many respiratory infection. The objective of this study is to identify the effective phytochemical of Ocimum sanctum (Tulsi) capable to curing Pneumoniae like Bronchitis Molecular docking Method. The phytochemical Chicoric acid acid can effectively deactivate Glyceraldehyde 3-phosphate Dehydrogenase enzyme thereby interrupting the life cycle of the pathogenic organism [6]. Considered as a powerful adaptogen, tulsi has an unique combination of pharmacological activities that advance prosperity and versatility [8].

This docking analysis study suggests that, Chicoric acid acid phytochemical extracted from *Ocimum sanctum* (Tulsi) can be used as a potent molecule against *A. xylosoxidans*. It can be used as a basic approach to design against this emerging infectious pathogenic infection. Further in- vivo and in-vitro studies is necessary for the further extension of drug discovery studies. Using the computational biology as well as bio-informatic tool for the designing of drug is a better approach to limit the duration and investment price. In the recent discovery of drug, when the protein-ligand is bounded to a protein receptor it is found that, the docking has a vital role in the prediction of the orientation of the ligand using the shape and electrostatic interactions specify it. Additionally, the Van der Waals interactions leads to an important role to Coulombic interaction and the hydrogen bonds formation. Together, all these interactions are calculated to docking score, generally represents the binding potentiality (Alberg and Schreiber 1993,[2].

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REFERENCES

1. StobbelaarK, HoorenbeeckKV, Lequesne M, DooyJD, Ho E,Vlieghe E, Margaretha Ieven, Stijn Verhulst. Sepsis Caused by Achromobacter Xylosoxidans in a Child with Cystic Fibrosis and Severe Lung Disease. 2016; 17:562-6.
2. Pagadala NS & Syed K & Tuszynski J. Software for molecular docking: a review. (2017) 9:91–102.
3. AmorimA, Bento J, Vaz AP, Gomes I, Gracia J,Hespanhol V, Marques A. Bronchiectasis: A retrospective study of clinical and aetiological investigation in a general respiratory department. 2015; 22(1):5-10.
4. HansenCR, Pressler T, Nielsen KG, Jensen PO, BjarnsholtT, HøibyN. Inflammation in *Achromobacter xylosoxidans* infected cystic fibrosis patients. 2010; 9: 51–58.
5. Marsac C, Berdah L,Thouvenin G, Sermet-Gaudelus I,Corvol H. *Achromobacter xylosoxidans* airway infection is associated with lung disease severity in children with cystic fibrosis. 2021;7: 1-6.
6. Palai B,Patra BP, Ray S,Swain S, Bhattacharyay D. Cardamom Derived Phytochemicals against Mycoplasma pneumonia Causing Bronchitis. 2020; 136-139.
7. Bjarnsholt T, Jensen PO, Fiandaca MJ, Pedersen J, Hansen CR, Andersen CB, et al. Pseudomonas aeruginosa biofilms in the respiratory tract of bronchitis patients. Pediatr Pulmonol. 2009;44(6):547–58.
8. Marc Maurice Cohen. Tulsi - *Ocimum sanctum*: A herb for all reasons. J Ayurveda Integr Med. 2014; 5(4): 251–259.
9. Barragán EP, Pérez JS, Corbella L, Orellana MÁ, Fernández-Ruiz M. Achromobacter xylosoxidans bacteremia: clinical and microbiological features in a 10-year case series. *Rev Esp Quimioter.* 2018; 31:268–273.

10. N.Hoyle,P.Zhvaniya,N.Balarjishvili,D.Bolkvadze,L.Nadareishvili,D.Nizharadze,J.Wittmann,C.Rohde, M. Kutateladze. Phage therapy against *Achromobacter xylosoxidan*s lung infection in a patient with cystic fibrosis: a case report. 2018;169(9):540-542.
11. Yabuuchi E, Oyama A. Achromobacter xylosoxidans n. sp. from human ear discharge. Jpn J Microbiol 1971; 15:477-81.
12. Rodrigues CG, Rays J, Kanegae MY. Native-valve endocarditis caused by Achromobacter xylosoxidans: a case report and review of literature. Autops Case Rep 2017; 7:50-5.
13. Behrens-Muller B, Conway J, Yoder J, Conover CS. Investigation and control of an outbreak of Achromobacter xylosoxidans bacteremia. Infect Control Hosp Epidemiol 2012; 33:180-4.
14. Park JH, Song NH, Koh JW. Achromobacter xylosoxidans keratitis after contact lens usage. Korean J Ophthalmol 2012; 26:49-53.
15. Tena D, Gonzalez-Praetorius A, Perez-Balsalobre M, Sancho O, Bisquert J. Urinary tract infection due to Achromobacter xylosoxidans: report of 9 cases. Scand J Infect Dis. 2008; 40:84-7.
16. Davies JC, Rubin BK. Emerging and unusual gram-negative infections in cystic fibrosis. Semin Respir Crit Care Med 2007; 28:312-21.
17. Edwards BD, Greysson-Wong J, Somayaji R, Waddell B, Whelan FJ, Storey DG, et al. Prevalence and Outcomes of Achromobacter Species Infections in Adults with Cystic Fibrosis: a North American Cohort Study. J Clinical Microbiol 2017; 55:2074-85.
18. Somayaji R, Stanojevic S, Tullis DE, Stephenson AL, Ratjen F, Waters V. Clinical Outcomes Associated with Achromobacter Species Infection in Patients with Cystic Fibrosis. An Am Thora Soc 2017; 14:1412-8.
19. Mahenthiralingam E. Emerging bronchitispathogens and the microbiome. Paediatr Respir Rev 2014;15 Suppl 1:13-5.
20. Trancassini M, Iebba V, Citera N, Tuccio V, Magni A, Varesi P, et al. Outbreak of Achromobacter xylosoxidans in an Italian Bronchitiscenter: genome variability, biofilm production, antibiotic resistance, and motility in isolated strains. Front Microbiol 2014; 5:138.

Table: 1. Kcal/mol value with dist from best mode

mode	Affinity (kcal/mol)	dist from best mode	
		rmsd l.b	rmsd u.b.
1	-8.2	0.000	0.000
2	-8.1	6.388	10.256
3	-7.6	0.788	8.699
4	-7.5	17.436	21.123
5	-7.5	1.592	9.263
6	-7.4	6.162	8.449
7	-7.4	7.452	12.081
8	-7.4	13.498	16.426
9	-7.4	18.949	22.089

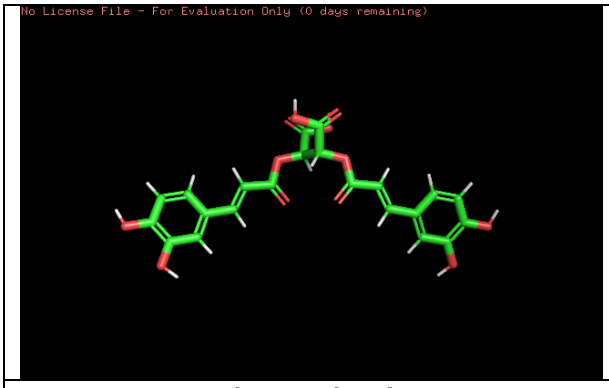

Figure 1: Ligand

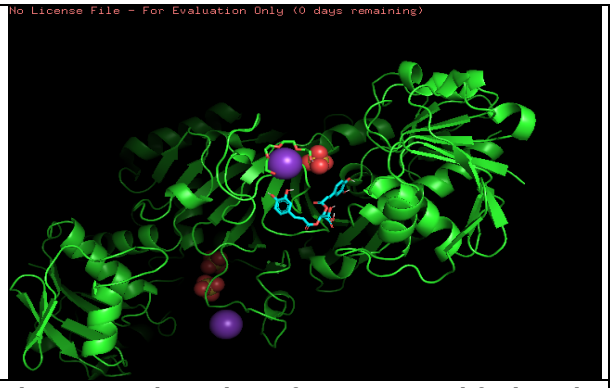

Figure 2:Active site of PDB ID-1obf (Protein Glyceraldehyde 3-phosphate Dehydrogenase from *Achromobacter xylosoxidans* with *Ocimum sanctum* (Tulsi)

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RESEARCH ARTICLE

Multi-Component Coupling Reactions for Diversity-Oriented Synthesis

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ABSTRACT

Chemistry as central technological know-how is facing a progressively increasing demand for brand spanking new chemical entities (NCE). Innovative answers, in all sorts of disciplines that rely on chemistry, require new molecules with specific residences, and their societal consequences are fundamental and pioneering. However, NCE now not the most effective call for a sensible structural space but additionally their feasibility poses challenges to artificial chemists. Nowadays the query of how to perform a synthesis has become most vital. Several novel methods, catalysts, and reagents were advanced to enhance organic synthesis. Synergistic results among reactions, reagents, and catalysts can cause minor heats of response and arise as an inherent result of multicomponent reactions (MCRs) and their extensions. They allow syntheses to be done at a low power level and the number of synthesis steps to be appreciably reduced in assessment with 'classical' two-thing reactions, pleasing the policies of Green Chemistry.

Keywords: MCRs, Green Chemistry, One-pot, Passerini, Isocyanide

INTRODUCTION

Multicomponent reaction (MCR) comprises one of the greatest effective forms in modernized synthetic organic chemistry because they have all components that provide an excellent synthesis [1]. A multicomponent reaction is a method in which three or more handily usable constituents are linked together in an individual reaction boat to

develop a final product demonstrating elements of all inputs and so attempts better potentialities for molecular diversity per step with a lowest synthetic time and effort. An MCR is a masking mechanism, a series of simple steps granting to a plan in which consecutive conversations are driven by the functionalities created in the prior step. MCRs comprise a particularly interesting synthetic approach since they administer easy and fast connection to extensive libraries of organic compounds with different substitution motif. While MCRs are a one-pot response, they are not difficult to execute than multistep synthesis. Connected with high-throughput athenaeum screening, this blueprint was a crucial advancement in drug discovery in the framework of hasty recognition and the escalation of biologically effective lead compounds. Libraries of small molecules are possibly the greatest wanted class of promising drug contestants, as regular peptides and oligonucleotides have disadvantages as the bio available remedy [2]. In the drug discloser operation, MCR proposes abundant merits over conventional paths. With only a restricted number of chemists and technicians, a higher scaffold synthesis plan can be attained in a period of a shorter time. With one-pot reactions, every synthesis method (weighing of reagents, the addition of reagents, reaction/time control) and work-up process (quenching, extraction, distillation, chromatography, weighing, and analysis) demands to be achieved only one time, in comparison to multi-step synthesis.

MCRs are adaptable with a solution aspect path, in this manner, permitting smooth auditing, and they are conveniently susceptible to computerization. Additionally, every scaffold is extendable from a small library to a larger library of compounds. Hence, "hit-to-lead" developments are usually polished freely and immediately. Positive physicochemical properties can be assembled into a library, e.g. lipophilicity and aqueous solubility, molecular weight, numbers of hydrogen donors and acceptors, and the number of rotatable bonds, likewise the polar surface area. Certainly, scale-up is repeatedly attainable from a preclinical lab-scale(mg, gram) to clinical preparatory quantity (kg) adopting the identical class of chemistry? Drug compounds developed from MCR are persuasive which, is the commitment of an hour [3]. Multicomponent reactions (MCRs) are notably fine adapted for multiplicity-oriented synthesis [4,5,6] and the fundamental influence originating from their briefness builds them still more effective for library synthesis proposed at executing structure-activity relationship (SAR) investigations of drug-like molecules, which are an important factor of the research achieved in pharmaceutical and agrochemical industries [7,8]. For all these senses, the advancement of Modern multicomponent reactions are briskly enhancing one of the borderlines of organic synthesis. Although a wide sector of the work grown in this territory is directed on reactions utilizing isonitriles as one of the important offset materials and superior to peptide-like structures, current years have endorsed a stable development in the growth of MCRs that edge precisely to heterocycles [9,10,11,12] the largest crucial class of compounds in the growth of bioactive compounds. Currently, there has been rising interest with attention to the rigid constitution on the perpetuation of “greenness” in synthetic alley and methods [13]. Green chemistry firmly important chemical research and there is a demand for the employment of ‘greener’ reaction environment [14]. Resilient organic solvents lot to over 85% of mass usage in conventional chemical producing methods, and on account of restoration ability is at a great distance from adequate, they are dominant subscribers to environmental contamination [15]. To get rid of organic solvents from the chemical methods, a substantial condition of green chemistry refers to the withdrawal of fickle organic solvents or their reinstatement by non-combustible, non-resilient, non-lethal and low cost “green solvent”[16].

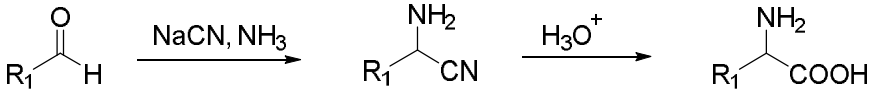
Multicomponent reactions [17] (MCRs) have been used very successfully to construct new heterocyclic molecules with relevant biological activity. These remarkable handy protocols have been known for over 150 years and occupy a central position in synthetic organic methodologies [18]. Multicomponent reactions can be defined [19] as “The reactions where more than two reactants combine sequentially, to give highly selective products that retain the majority of the atoms of the starting materials’’. MCRs hold an advantageous position in modern organic synthesis because it provides the best way to generate complex molecules with various functional substituents from three or more readily available starting materials in a limited step [20]. These reactions have frequently been used to create eco-friendly, cost-effective, and convenient chemical methods for the discovery of new chemical entities necessary for industries [21]. Moreover, multicomponent reactions are usually one-pot synthesis, so, it is easy to accomplish the reactions with a good yield as compared to multistep synthesis [22]. The most important features of MCR’s are

numerous bond formations in a single step without changing the reaction conditions by adding more reagents. As a result, it minimizes the formation of by-products in the reactions.

History of MCRs

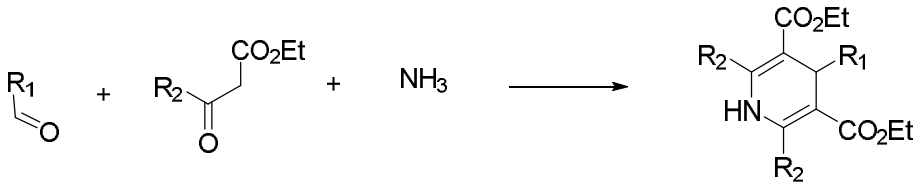
There have been many records on MCRs so far, and common illustrations are explained as below.

Strecker reaction
(Three-component reaction: 3CR)

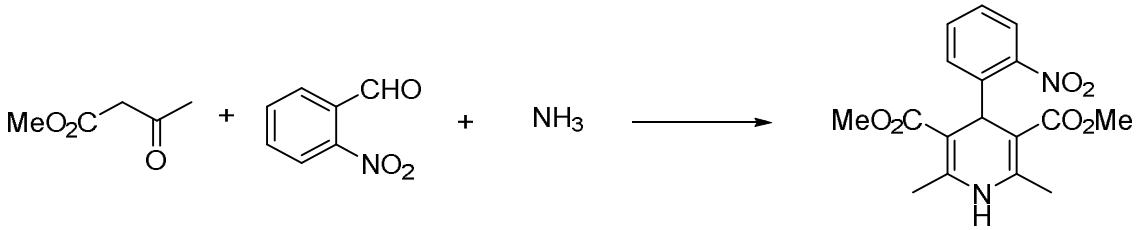


In 1850, this reaction was disclosed by A. Strecker. It is acutely acclaimed as the synthesis of α-amino acids. This reaction is a multi-component coupling reaction (MCR) which consists of three constituents, aldehyde, hydrogen cyanide, and ammonia as substrates, and is perceived as the world's first MCR [23].

Hantzsch dihydropyridine synthesis (3CR)

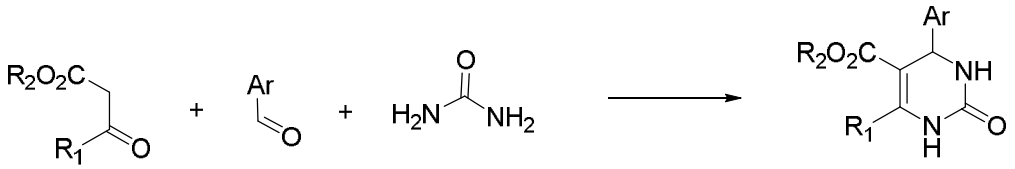


In 1881, A.R Hantzsch explained this reaction and is the very famous three-component MCR, which gives 1,4-dihydropyridine derivatives utilizing 2moles of β-keto esters, aldehydes, and ammonia [24]. A calcium channel blocker, "Nifedipine:" was synthesized by this reaction [25,26,27,28].



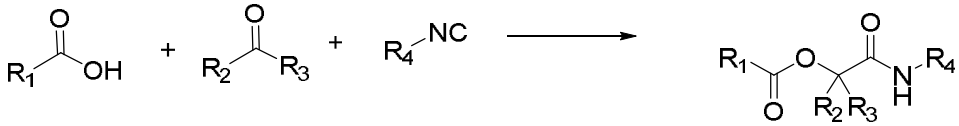
Nifedipine

Biginelli reaction (3CR)



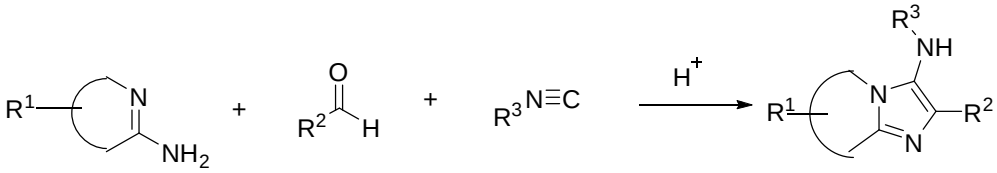
An Italian chemist, P.Biginelli has announced the three-component reaction adopting β-keto esters for example ethyl acetoacetate, aromatic aldehydes for example benzaldehyde, and urea (e.g. thiourea)in the existence of acid catalyst (Lewis acids or Bronsted acids), allowing dihydropyrimidone derivatives [29,30]. Dihydropyrimidones have been rewarded more concentration on account of their miscellaneous bioactivities. Pharmaceuticals discovered antitubercular agents by utilizing this reaction and it has been stated as below [31,32].

Passerini reaction (3CR)



An Italian chemist, M. Passerini *et al*., has illustrated the three-component-coupling reaction in 1921, utilizing carboxylic acids, aldehydes, and isonitriles, allowing α-acyloxy amides [33]. The Passerini reaction further has been used into pharmaceutical research,Hulme*et al*. have expressed the athenaeum synthesis of unique norstatine derivatives posturing benzimidazole moieties [34].

Grobcke-Blackburn-Bienayme reaction [35,36,37,38] (3CR)



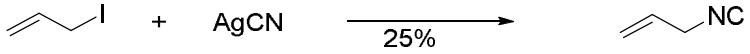
The three-component MCR reaction utilizing aldehydes, isonitriles, and α-aminoazines like 2-aminopyridine or 2-aminoimidazole in the existence of acid catalyst. This reaction is suitable for the preparation of fused nitrogen-containing aromatic compounds.

Isocyanides

Isocyanides (isonitriles) described for age the only class of stable organic compounds with a regularly divalent carbon. By cause of its reactivity, the isocyanide group varies mostly from other functional groups. Nearly all commercially feasible isocyanides are unstable and bear this disgusting, stinging, unpleasant smell. Due to this type of smell isocyanides have been examined as promising non-fatal weapons [39].

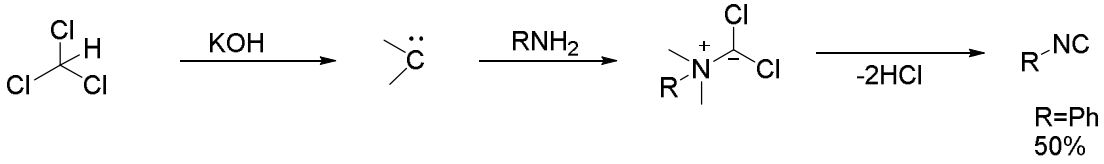
Synthesis of Isocyanides [40]

Lieke synthesis of isocyanide.



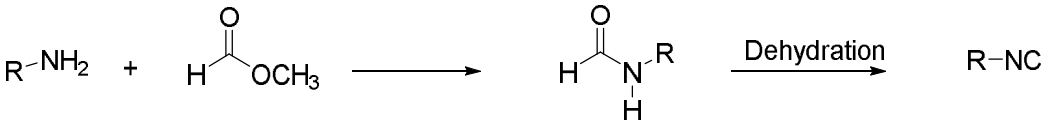
In 1859 Like has first synthesized Isocyanides [41] Who was amazed to achieved compound with a terrible smell, which evaporated by continued heating. In 1869, Gautier [42] verified that such allylation gave the allyl isocyanide and displayed the isomeric connection between isocyanides and nitriles. (Hydrolysis of isocyanide gave formamide instead corresponding carboxylic acid)

Hoffmann synthesis of isocyanide (Carbylamine method)



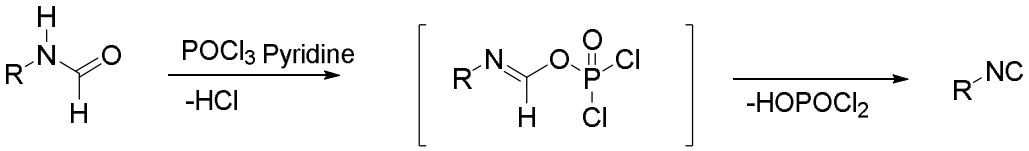
In 1867, Hofmann [43] created a new way to isocyanides via condensation of the primary amine with a dichlorocarbene, produced in situ by heating chloroform with potassium hydroxide. However, this method deteriorates from a need for reproducibility, small yield, and problems of separation of isocyanides from amines.

Synthesis of isocyanide by dehydration of formamide

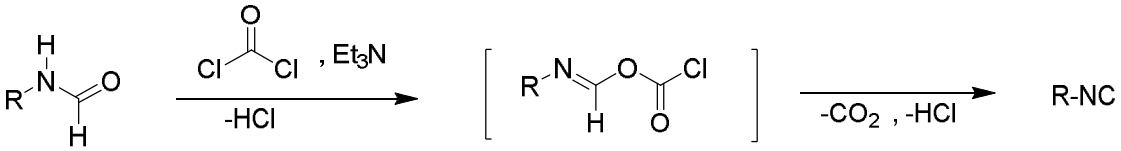


Because of painful admission, only a few isocyanides have been known for one century and rather few types of reactions have been characterized. Ivar Ugi has shared very meaningfully in isocyanide chemistry. Ugi developed the formation of isocyanide using dehydration of N-monosubstituted formamide; these formamides can be produced from primary amines and methyl or ethyl formate or formic acids.

Synthesis of Isocyanide by dehydration of formamide.



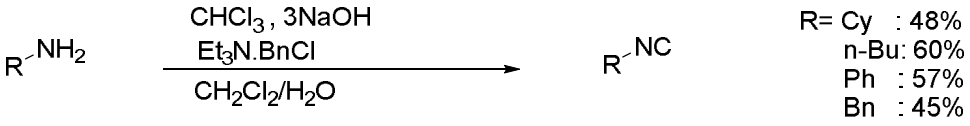
(a) Using POCl3 as dehydrating agent



(b) Using Phosgene as a dehydrating agent

Miscellaneous dehydrating agents can be employed (for example $SOCl_2$, PBr_3, P_2O_5, $POCl_3$, $(CO)_2Cl_2$) in the attendance of a base like triethylamine, pyridine, diisopropylethylamine. Ugi addresses more than 230 isocyanide synthesis. A few of them are displayed below.

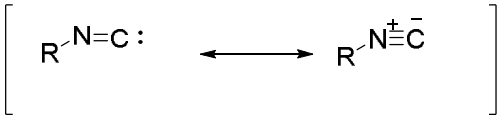
Hoffman Synthesis of isocyanide.



Ugi also upgraded the Hoffman method of carbylamines by performing it in a biphasic medium- the combination of dichloromethane and water in the presence of a PTC [44] (phase transfer catalyst). In this procedure, the strike of the primary amine on dichlorocarbene is more choosy and the procedure is high yielding (~70% after purification) and more reproducible.

Reactivity of isocyanides

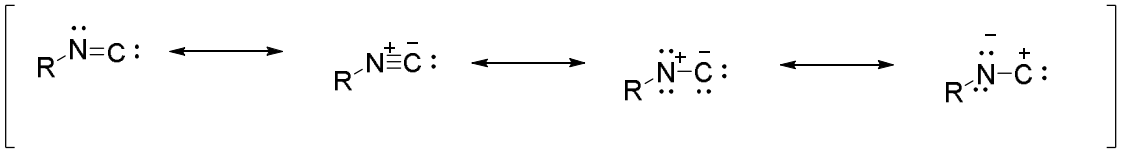
Resonance structure of isocyanide



1a 1b

Isocyanides are studied as resonance modes between divalent carbon and zwitterions 1a and 2b, the carbon atom of the isocyano group displays a carbine-like reactivity that is mirrored in the resonance form 1a. Oppositely, the linear structure of isocyanides is well pictured by the dipolar resonance structure 1b.

Valence bond study of resonance structures of isocyanides.

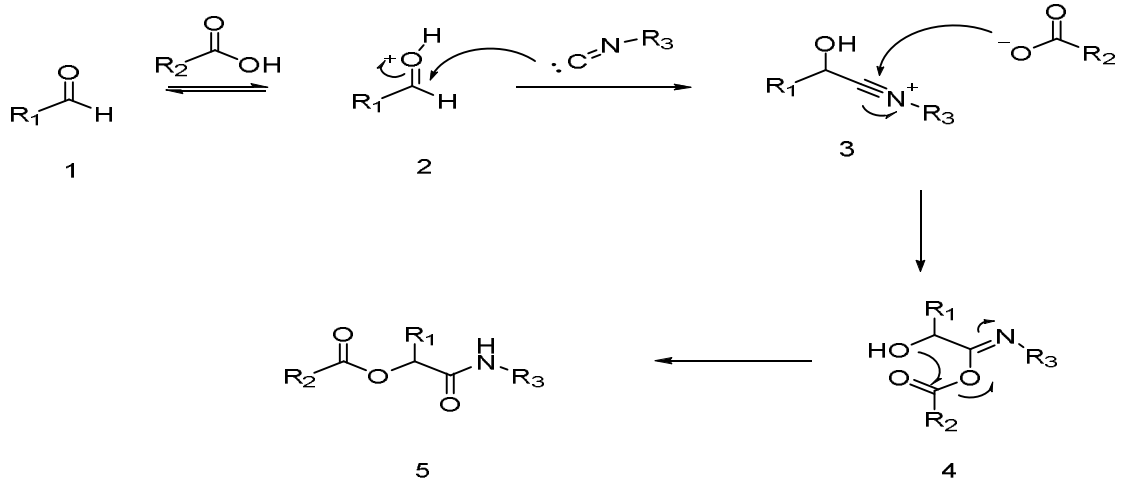


Valence Bond Theory for methyl isocyanide [45] demonstrates that the carbine modesexist at around fifty percent whereas the zwitterionic form reports for around thirty percent of structures and the resting twenty percent forms being more complicated. Thus isocyanides are the linear cause of its geometry inflates the resonance between the carbon and zwitterionic modes.

Under the basic operation, Isocyanides are steady (they are generally prepared in the basic environment), although they are completely sensitive to acids. In the attendance of aqueous acidic solutions, isocyanides respond to deliver the resemble formamides, and acidic hydrolysis is a normally useful technique to take off the unpleasant odor of isocyanides. Highest isocyanides polymerize in the existence of acids [46]. Isocyanide chemistry is typified by three properties: the alpha-acidity, the alpha-addition, the formation of radicals.

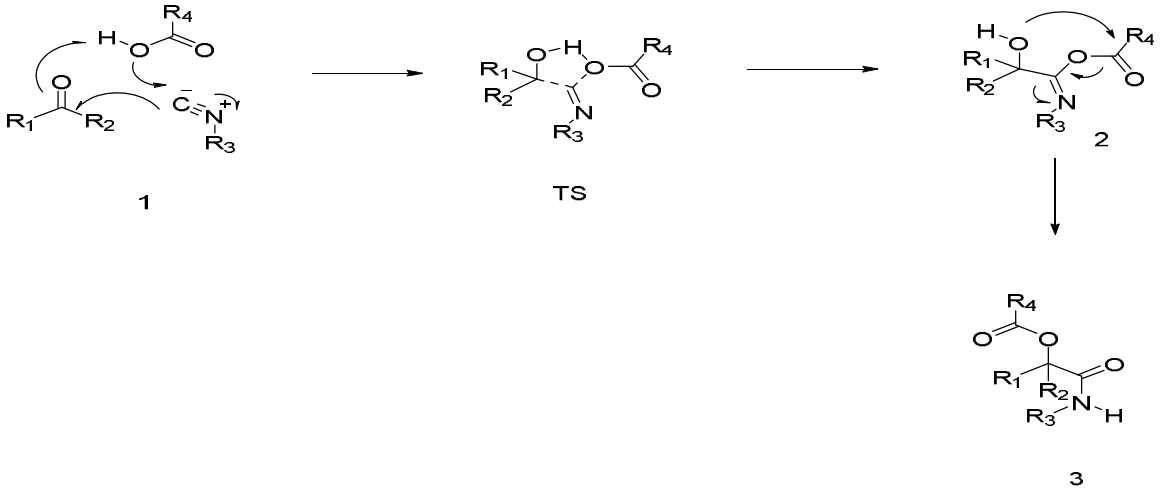
Reaction mechanism

Two offbeat reaction routes have been contemplated.



Scheme: 1. Ionic mechanism

The reaction proceeds by protonation of the carbonyl carbon in polar solvents such as water or methanol followed by the nucleophilic addition of the isocyanide to give the nitrillium ion 3. The addition of carboxylate gives intermediate 4. Acyl group transfer and amide tautomerization give the desired ester 5. Concerted mechanism [47]. The concerted mechanism is expected in non-polar solvents and at high concentrations.



Scheme:2. concerted mechanism

This system comprises a trimolecular reaction between among the isocyanide (R-NC), the carboxylic acid and the carbonyl in an array of nucleophilic additions. The TS is described as a 5-membered ring with partial covalent or double bonding. The second step is an acyl transfer to the adjacent hydroxyl group. The reaction continues in almost non-polar solvents and the kinetics of the reaction relies upon all three reactants.

CONCLUSION

In this newsletter, the use of different multicomponent reactions (MCRs) for the synthesis of heterocyclic compounds has been reviewed. This overview demonstrates the artificial potential of multicomponent reactions for the development of heterocyclic molecules. MCRs have the capability to generate libraries of small molecules with excessive stages of complexity and variety. Recently, an effective approach for maximizing those functions has been delivered. In the MCR reaction, constructing blocks possessing orthogonal functionalities are employed, and the MCR is sequenced with extra elaboration or cyclization tactics that substantially increase the molecular complexity and diversity of the goods. The maximum powerful synthetic methodologies stand up whilst the practical components are properly optimized, and the complete sequence may be finished as a one-pot operation. Furthermore, the most various products are produced when divergent response pathways will be opened by using variations in the structures of the substrates for the MCR, or with the aid of the selection of the reagents or reaction conditions. Herein, current studies geared toward designing such protocols and their programs to the synthesis of numerous assemblies of medicinally relevant heterocycles have been reviewed.

REFERENCES

1. Wander P.A.(2014) Nat. Prod. Rep., (31) 433-440.
2. Jieping z., Qian w., Meixing w. (2015) Multicomponent reaction in organic synthesis, wiley-vch, 8.
3. Hulme C., Gore V. (2003) Curr. Med. Chem., (10) 51-80.
4. Spring, D. R. (2003) Org. Biomol. Chem., (1) 3867.

5. Burke M.D., Schreiber S.L. (2004) Angew. Chem., Int. Ed., (43) 46.
6. Spandl R.J., Bender A. Spring, D. R. (2008) Org. Biomol. Chem., (6)1149.
7. Weber L. (2002) Curr. Med. Chem., (9)2085.
8. Hulme C., Gore V. (2003) Curr. Med. Chem.,(10)51.
9. Sapi, J., Laronze Y. (2004) Arkivoc,, (vii)208.
10. D'Souza D. M. (2007) Mu¨ ller. T. J. J. Chem. Soc. Rev., (36)1095.
11. Isambert N., Lavilla R. (2008) Chem.–Eur. J.,(14)8444.
12. Sunderhaus J.D., Martin S.F. (2009) Chem.–Eur. J.,(15)1300.
13. Tundo P., Anastas P., Black D.S., Breen J., Collins T., Memoli S., Miyamoto J., Polyakoff M., Tumas W. (2000) Pure Appl.Chem., (72)1207.
14. Alfonsi K., Colberg J., Dunn P.J., Fevig T., Jennings S., Johnson T.A., Kleine H.P., Knight C., Nagy M.A., Perry D.A., Stefaniak M. (2008) Green Chem., (10) 31.
15. Constable D.J., Gonzalez C.J., Henderson R.K.(2007) Org. Process Res. Dev.,(11) 133.
16. Reinhardt D., Ilgen F., Kralisch D., Konig B., Kreisel G.(2008) Green Chem.,(10)1170.
17. Pandya K.M., Desai P.S. (2020) Rasayan J. Chem., 13(2)1054-1062.
18. González C.J., Constable D.J.C.(2011) Green Chemistry and Engineering, John Wiley & Sons, Inc.: New Jersey.
19. Domling A., Ugi I. (2000) Angew. Chem. Int. Ed. Engl., 39, 3168.
20. Orru R.V.A., de Greef M. (2003) Synthesis.,1471.
21. Cioc R.C., Ruijter E., Orru R.V.A.(2014) Green Chem., (16)2958.
22. Bienayme H., Hulme C., Oddon G., Schmitt P.(2000) Chem. Eur. J., (6)3321.
23. Strecker A.(1850) Ann., (75) 27.
24. Hantzsch A. (1881) Ber., 14)1637.
25. Alajarin R., Vaquero J. J., Navio J. L.G., Builla J.A. (1992) Synlett, 297.
26. Alajarin R., Jordan P., Vaquero J.J., Builla J.A.(1995) Synthesis, 389.
27. Khadilkar B.M., Chitnavis A.A.(1995) Ind. J. Chem., (34B) 652.
28. Khadilkar B.M., Gaikar V.G., Chitnavis A.A. (1995) Tetrahedron Lett.,(36) 8083.
29. Biginelli P. (1891) Ber., (24)1317.
30. Biginelli P. (1891) Ber., (24) 2962.
31. Trivedi A.R., Bhuva V.R., Dholariya B.H., Dodiya D.K., Kataria V.B., Shah V.H. (2010) Bioorg. Med. Chem. Lett.,(20) 6100.
32. Virsodia V., Pissurlenkar R.R.S., Manvar D., Dholakia C., Adlakha P., Shah A.E. (2008) Coutinho, Eur. J. Med. Chem., (43)2103.
33. Passerini M.(1921) Gazz. Chim. Ital., (51)181.
34. Shaw A.Y., Medda F., Hulme C. (2012) Tetrahedron Lett., (53)1313.
35. Gröbke K., Weber L., Mehlin F.(1998) Synlett, 661.
36. Blackburn C., Guan B., Shiosaki K., Tsai S.(1998) Tetrahedron Lett.,(39)3635.
37. Bienaymé H., Bouzid K. (1998) Angew. Chem., (110)2349.
38. Bienaymé H., Bouzid K. (1998) Angew. Chem. Int. Ed.,(39) 2234.
39. Pirrung M., Ghorai S., (2009) Ibarra-Rivera T. J.Org.Chem., (74) 4110-4117.
40. Ugi I., Meyr R., (1958) Angewandte Chemie.,(112)316-321.
41. Lieke W. (1859) Justus Liebigs Ann. Chem.,(112)316-321.
42. Gautier A. (1869) Justus Liebigs Ann.Chem., (146)119-124.
43. Hoffmann A.W.(1867) JustusLiebigs Ann.Chem., (144)114-120.
44. Weber W., Gokel G., Ugi I.(1972) Angew.Chem., (84)587-587
45. Ramozzi R., Cheron N., Braida P., Fleurat P.(2012) New J.Chem., (36)1137-1140.
46. Van B.(1983) A. Macromolecules, (16)1679.
47. Domling A., Ugi I.(2000) Angew.Chem.Int.Ed.Engl., (39)3168-3210.

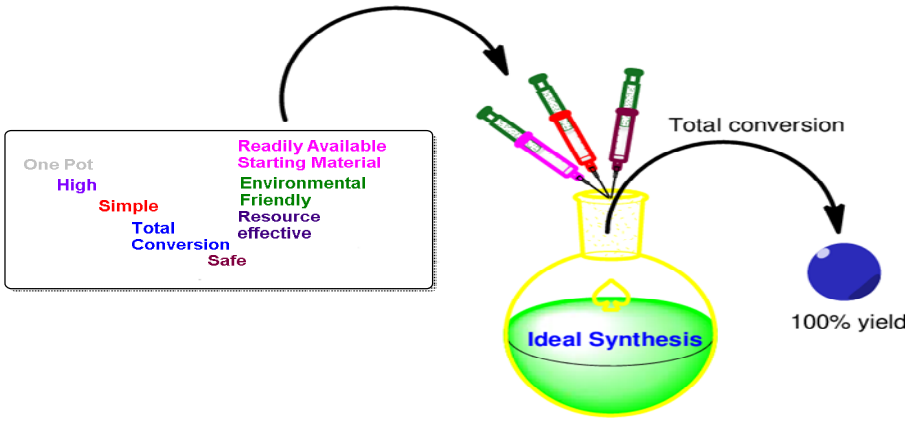


Fig.1. Ideal Synthesis

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REVIEW ARTICLE

A Brief Review on Synergistic Antimicrobial Activity of Some Bioactive Phytochemicals

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ABSTRACT

Since ancient times plants are considered as the major source of novel drug molecules and have served a great extent in the treatment of different infectious diseases. The secondary plant metabolites are having miraculous healing properties and reported to shown potent therapeutic response when used in combination drug therapy. The prime objective of this review is to summarize the concept of drug combination with special emphasis on the synergistic interaction between pant derived bioactive phytochemicals with commercially available antimicrobial agents. The study further contributes to assessing their role, importance, and applicability in the management of different diseases condition. The review significantly focuses on different aspects of synergistic antimicrobial activities, mechanism of synergism and current status of researches in the field. The study was carried out based on an extensive literature survey based on the hypothesis, that secondary metabolites derive from plants possess miraculous therapeutic activities. This article contains concise information on the most commonly used bioactive phytochemicals having potent antifungal and antibacterial effects.

Keywords: antimicrobial & antifungal, bioactives, medicinal plants, Phytochemicals, synergestic

INTRODUCTION

Since the early beginning, our ancestors effectively use natural resources as the sole means to heal different infections and injuries. In fact, during the past few decades' plant-derived bioactive materials played an incredible role in modern drug discovery. The traditional system of medicine like Ayurveda, Charaka Saṃhita around 900BC described a list of 341 plants derived medicine. Similarly, Sushruta Samhita around 600BC described 395 medicinal plants and traditional systems of Chinese medicine around 350BC describe 247 medicinal plants and 157 combinations of plant-derived medicines. This valuable literature ensures that the plants or plant-derived

phytochemicals have miraculous healing capacities against most challenging diseases of society [1]. Till the early 1900s rational use of the majority of plants derive bioactive phytochemicals are remained untouched due to the deficiency of modern research facilities. The introduction of modern research techniques and research tools provides a huge scope to explore the unknown medicinal values of designate phytochemicals or bioactive molecules [2]. Combination drug therapy is considered a promising approach to treat complex diseases such as cancer, fungal and bacterial infections, inflammation and many more [1-3]. However, the phenomenon of combined drug interactions is unpredictable. The most commonly distinguished interaction between two drugs may be technically known as synergism, antagonism, and summation or additional action. Where synergism may be explained as a phenomenon in which the pharmacological action of one therapeutically active molecule is increased in the presence of other molecules. The therapeutic activities of plants derive phytochemicals and their role in synergism may differ significantly [3]. Despite several controversies, today it is possible to make an intense remark on the synergistic activity of therapeutically active phytochemicals in combination with synthetic molecules. A modern concept like "Isobole method of Berenbaum", highly efficient technology like Omic technology, pharmacokinetic and pharmacodynamic methods of estimation, biochemical pathway analysis of combinations, etc. are the most effective tools for monitoring synergism. The reported literature shows that the mechanisms of synergistic activities may be of several types [1,4].Considering the above fact the present study has been designed to summarize the information about the effective combinations of natural and synthetic compounds that are more effectively antifungal action. Apart from that information related to types and its different mechanisms of action are also discussed in this study.

Concept of Synergism and Its role to manage infectious diseases

The term synergy comes from the Greek word "*synergos*", meaning "working together". The concept of synergy refers to the combination of two or more compounds that generate outcomes greater than the additive impact of those individual compounds. This outcome is the result of interactions, in which compounds enhance each other's performance towards achieving a desirable goal. However, if the interaction of bioactive agents or compounds leads to a result that is less than the sum of the individual effects will be designated as antagonism. To understand the role and importance of synergism, one should have a clear idea about the history as well as the present scenario of infectious diseases[5]. The available reports and literature describe that from the early beginning infectious diseases caused by pathogenic bacteria and fungi have remained the most devastative life-threatening issue for society.

Infectious diseases affect worldwide millions of people. As per the WHO report, approximately 50,000 peoples die per day throughout the world from these diseases. Hence, the discovery of antimicrobial agents such as antifungal & antibiotics was considered as a major achievement in the history of medical sciences [6]. As time proceeds, day-by-day new antimicrobial molecules were introduced in the market against different active pathogens. Since the last few decades, due to the irrational and abundant misuse of antimicrobials make the clinically important microbes more commanding against developed antimicrobial agents. Through a continuous effort by the leading scientists are already in the way to develop new innovative and effective molecules or drugs that can effectively manage infectious diseases [7].However, it is a fact that the invention of a new molecule takes time. Therefore, the lake of new effective molecules as well as pathogenic resistance on existing molecules makes this area a global health threat.

Several approaches have been tried to manage this situation, among this multidrug therapy concept were found comparatively more suitable for effective treatment of infectious disease [8, 9]. However, it has seen that the use of synthetic antimicrobials in combination at a higher strength, produces a high level of side effect and toxicity therefore rational use of such multidrug therapy is still under questionable issues. Since long, plant-derived bioactive or phytochemicals are used either in single or in combination, effectively and successfully against different life treating infectious diseases. Several studies have proposed that natural compounds are capable to potentiate the activity of existing antibiotics &antifungal. In this era, huge research has been carried out to explore medicinal values of different pant or plant derived products and the results indicate a future scope of rationale use of such bioactive materials will surely help to achieve pharmacological and therapeutic superiority.

Mechanism of synergism

The role of bioactive phytochemicals on modern therapy can only be justified through exact knowledge and understanding of the mechanisms involved behind the therapeutic activity. Based on the latest development in the field of classic pharmacological, molecular biological and
Clinical techniques, the mechanisms of synergism may be classified as follows:

Synergism due to Pharmacokinetic or physicochemical interaction: this type of interaction can modify the normal solubility, absorption rate, reabsorption rate and bioavailability of drugs [10].

Synergism due to Pharmacodynamics interaction: This type of interaction involves two drugs, which are exerting their activity by joining to the same receptor [11,12].

Synergism that resists resistance mechanisms against bacteria and fungi: In this type of interaction, the presence of one molecule may improve or facilitate the fundamental mechanism of another chemical entity, which results in a better therapeutic action. This kind of synergy happens when antibiotics combined with such chemical entities that can partly or completely suppress bacterial resistance mechanisms. For example combination penicillin with clavulanic acid (sulbactam or tazobactam) which successfully overcome penicillinase resistance due to three possible reasons:
(i) modification of active site .
(ii) modification antibiotic action due by enzymes .
(iii) Alteration in the efflux of antibiotics from the cell [1,10].

Synergistic multi-target effects: Synergistic Multi-target effects' means that single constituents may show combination affect not only one single target, but also several targets, and therefore may conjoin in an agonistic, synergistic way. Investigation of synergism describes the evidence of multi-targeting that affect gene expression at molecular level E.g. Methotrexate targeted a multitude of genes involves in apoptosis, mismatch repair, cell cycle control and stress response [1,10].

Most common methods for *In- Vitro* evaluation of synergy:

The accurate estimation of synergy between commercial drug molecules or between a drug and bioactive phytochemical based upon the results of in vitro testing is very essential. Several methods are used to detect synergy. In-depth understanding of molecular biology, implementation of new clinical and statistical tools for accurate estimation & introduction of modern technology like omic methods, gene microassay technology makes processes of in-vitro evaluation of synergy more accurate. However, as per an infectious disease concern, the checkerboard and time-kill curve methods are the two most easily and widely used techniques [13]. In case of the checkerboard method, microtitrater plates are used, where for multiple combinations of two antimicrobial agents in different concentrations (equal to, above, and below) their minimal inhibitory concentrations for the microorganism that are being tested. The combination in which the growth is completely inhibited is taken as the effective MIC for the combination [13,14]. On the other hand, the time-kill method assesses the bactericidal activity of the individual as well as the different concentrations of the combination of drugs as a function of time. The tubes containing individual as well as the combination of compounds with different concentrations ranging from one-quarter to twice the MIC for the bacterial strain are prepared as per the guideline of NCCLS and are incubated overnight. Aliquots of the samples from 0 h of incubation (reflecting the initial inoculum) and 24 h of incubation (reflecting exposure of bacteria to the compound) are estimated. If a 100-fold or greater decrease in colony count at 24 h by the combination of agents regarding the starting inoculum found, the phenomenon would be concluded as synergism [13–15]. E test is another method, which is effectively used for evaluation of synergy. It consists of two plastic strips coated with a continuous gradient of each of the compound on one side. The first compound strip is placed onto an agar plate for 1 h and then

removed, and the second compound strip is placed on top of the gradient left behind by the first. The MIC of the combination is taken as the value at which the two inhibition zones intersect [15].
Further, standardization of these techniques for routine laboratory testing is required for effective and accurate estimation of drug combinations

DISCUSSION

Combination drug therapy is not a new concept, however, innovative and rational use of phytochemicals to develop safe and effective combination therapy may be the future of infectious disease management. Although the term synergism may be considered as a type of combination therapy, where the presence of one component effectively improves the activity of others, the mechanism of these therapies may be of several types. The introduction of modern and improved technology, statistical tools and in-depth knowledge of molecular mechanisms involves may play a key role to understand the phenomenon of synergism and its types. In this article, we have tried to present a summary of the most effective bioactive compounds that are often used in the management of infectious disease along with their possible reported path of mechanism action. Again detailed information about documented proof of synergistic effects of some plant derived bioactive molecules in combination with synthetics drugs is also discussed.

Despite enormous efforts, proper information on the molecular mechanism behind the effectiveness of most of the bioactive phytochemicals is found to be limited. Therefore, a proper understanding of the mechanism will be the key deciding factor. As per as activity of plant-derived phytochemical study concern, in most of the cases, the appropriate molecular mechanisms are still undefined. Although researchers have reported that different plant extracts show significant therapeutic effects but still lead molecules responsible for the shown activities or path of the mechanism are yet to be explored. Safe and effective therapy from plant derive bioactive mainly depends on the toxicity profile of molecule, Therefore, the concentration of phytochemicals in a combination and frequency of use of those combinations are the two key factors that are to be followed strictly to get optimum benefit. Development of resistance against available synthetic antibacterial & antifungal by pathogenic stains is the biggest issue of this era, which can be easily counteracted with the proper scientific investigation & explanation of the holistic approach of traditional medication & effective utilization of natural resources.

CONCLUSION

The present review highlights several aspects of synergism and the role of plant derive bioactive phytochemicals to develop effective drug combination for the management of infectious diseases. From the above discussion, it can be concluded that bioactive phytochemicals obtained from different medicinal plants shows promising healing capacity and may effectively contribute different diseases. The clear understanding of molecular mechanism of such bioactive compounds will surely enable a better scope for the disease management. Although the rational designing of drug combination with synergistic effects seems to be a challenging task, but it may became a potential treatment stratetigy for the future.

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CONFLICT OF INTEREST

NIL

REFERENCES

1. Gudrun Ulrich-Merzenich, D Panek. H Zeitler HV& HW. Drug development from natural products : Exploiting synergistic effects. Indian J. Exp. Biol., 2010; **48**,208–219.
2. Calixto A B. The role of natural products in a modern drug discovery, Anais da Academia Brasileira de Ciências, 2019; 91(Supply-3), 1–7.
3. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine, 2008; **15**, 639–52.
4. Ma XH, Zheng CJ, Han LY, Xie B, Jia J, Cao ZW, Li YX, Chen YZ. Synergistic therapeutic actions of herbal ingredients and their mechanisms from molecular interaction and network perspectives. Drug Discov. Today, 2009; **14**, 579–88.
5. Rani A, Jain S, Dureja P, Kumar R, Kumar A. Synergistic interaction between synthetic and natural products: a promising tool for the development of environmentally safe potent antimicrobial agents, International Journal of Chemistry. 2015; 7(2), 170-176.
6. Roy A, Choudhury A, Bahadur S, Saha S. Phytoconstituents based mucoadhsive antifungal vaginal formulation : An effective and innovative approch. 2016; Biosci. Biotechnol. Res. Commun., **9**, 694–701.
7. Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria. Clin. Microbiol. Rev., 2012; **25**, 450–70.
8. Anshul Nigama, Divya Gupta b AS. Treatment of infectious disease : beyond antibiotics. Microbiol. Res., 2014; **169**, 643–51.
9. Wagner, H GU-M. Synergy research : approaching a new generation of phytopharmaceuticals. J. Nat. Remedies, 2009; **9**, 121–41.
10. Tallarida RJ. Drug Synergism : Its Detection and Applications. J. Pharmacol. Exp. Ther., **298**, 2001; 865–72.
11. Chen D, Liu X, Yang Y, Yang H, Lu P. Systematic synergy modeling : understanding drug synergy from a systems biology perspective. BMC Syst. Biol., 2015; **56**, 1–10.
12. Sopirala MM, Mangino JE, Gebreyes WA, Biller B, Bannerman T, Balada-Llasat JM, Pancholi P. Synergy testing by E-test, microdilution checkerboard, and time-kill methods for pan-drug-resistant Acinetobacter baumannii. Antimicrob. Agents Chemother., 2010; **54**, 4678–83.
13. Orhan G, Bayram A, Zer Y, Balci I. Synergy tests by E test and checkerboard methods of antimicrobial combinations against Brucella melitensis. J. Clin. Microbiol., 2005; **43**, 140–3.
14. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J. Pharm. Anal., 2016; **6**, 71–9.
15. Tsao SM, Yin MC. Enhanced inhibitory effect from interaction of curcumin with amphotericin B or fluconazole against Candida species. J. Food Drug Anal., 2000; **8**, 208–12.
16. Chung I, Kwon SH, Shim ST, Kyung KH. Synergistic antiyeast activity of garlic oil and allyl alcohol derived from alliin in garlic. J. Food Sci., 2007; **72**, 437–440.
17. Of E, Nanoemulsion S. Synergestic effect of some medicinal plants and antibiotics against few pathogenic bacteria. Int. J. Biol. Pharm. Res., 2012; **3**, 767–74.
18. Rabadia A, Kamat S, Kamat D. Antifungal Activity of Essential Oils Against Fluconazole Resistant Fungi. *Int J Phytomed*, 2012; **3**, 506–510.
19. Garcia-Gomes AS, Curvelo JAR, Soares RMA, Ferreira-Pereira A. Curcumin acts synergistically with fluconazole to sensitize a clinical isolate of Candida albicans showing a MDR phenotype. *Med. Mycol.*, 2012; **50**, 26–32.
20. Pyun MS, Shin S. Antifungal effects of the volatile oils from Allium plants against Trichophyton species and synergism of the oils with ketoconazole. Phytomedicine, 2006; **13**, 394–400.
21. Faria NCG, Kim JH, Gonçalves LAP, Martins MDL, Chan KL, Campbell BC. Enhanced activity of antifungal drugs using natural phenolics against yeast strains of Candida and Cryptococcus. Lett. Appl. Microbiol., 2011; **52**, 506–13.

22. Canturk Z. Evaluation of synergistic anticandidal and apoptotic effects of ferulic acid and caspofungin against Candida albicans. 2017; J. Food Drug Anal., **2**, 10–4.
23. Jayaraman P, Sakharkar MK, Lim CS, Tang TH, Sakharkar KR. Activity and interactions of antibiotic and phytochemical combinations against pseudomonas aeruginosa in vitro. Int. J. Biol. Sci., 2010; **6**, 556–68.
24. Roccaro AS, Blanco AR, Giuliano F, Rusciano D, Enea V. Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. Antimicrob. Agents Chemother., 2004; **48**, 1968–73.
25. Sakharkar MK, Jayaraman P, Soe WM, Chow VTK, Sing LC, Sakharkar KR. In vitro combinations of antibiotics and phytochemicals against Pseudomonas aeruginosa. *J. Microbiol. Immunol. Infect.*, **42**, 364–70 (2009).
26. Liu M-H, Otsuka N, Noyori K, Shiota S, Ogawa W, Kuroda T, Hatano T, Tsuchiya T. Synergistic effect of kaempferol glycosides purified from Laurus nobilis and fluoroquinolones on methicillin-resistant Staphylococcus aureus. Biol. Pharm. Bull., 2009; **32**, 489–92.
27. Lewandowska U, Gorlach S, Owczarek K, Hrabec E, Szewczyk K. Synergistic interactions between anticancer chemotherapeutics and phenolic compounds and anticancer synergy between polyphenols. Postepy Hig. Med. Dosw., 2014; **68**, 528–40.
28. Zhu Y, He W, Gao X, Li B, Mei C, Xu R, Chen H. Resveratrol overcomes gefitinib resistance by increasing the intracellular gefitinib concentration and triggering apoptosis, autophagy and senescence in PC9 / G NSCLC cells. Nat. Publ. Gr. 2015; 1–12.
29. de Oliveira DR akelly, Tintino SR elison, Braga MF laviana BM orais, Boligon AA ugusti, Athayde ML inde, Coutinho HD ouglas M, de Menezes IR ose A, Fachinetto R. In vitro antimicrobial and modulatory activity of the natural products silymarin and silibinin. Biomed Res. Int., 2015; **2**, 1–7.
30. Batista J, Neto DA, Rocha C, Campos RDS, Freitas DD De, Josino MA, Andrade LND De, Iury H, Magalhães F, Gaspar DM, De MO, Vitoriano H, Júnior N. Evaluation of Synergistic Effect of Kaurenoic Acid Derivatives with Fluconazole against Strains of Fluconazole-Resistant Candida parapsilosis. Int.J.Curr.Microbiol.App.Sci, 2015; **4**, 68–79.
31. Liu W, Li LP, Zhang JD, Li Q, Shen H, Chen SM, He LJ, Yan L, Xu GT, An MM, Jiang YY. Synergistic antifungal effect of glabridin and fluconazole. PLoS One, 2014; **9**, 1–10.
32. Kobayashi T, Kakeya H, Miyazaki T, Izumikawa K, Yanagihara K, Ohno H, Yamamoto Y, Tashiro T, Kohno S. Synergistic antifungal effect of lactoferrin with azole antifungals against candida albicans and a proposal for a new treatment method for invasive candidiasis. Jpn. J. Infect. Dis., 2011; **64**, 292–6.
33. Ettayebi K, El Yamani J, Rossi-Hassani BD. Synergistic effects of nisin and thymol on antimicrobial activities in Listeria monocytogenes and Bacillus subtilis. FEMS Microbiol. Lett., 2000; **183**, 191–195.
34. Reuk-Ngam N, Chimnoi N, Khunnawutmanotham N, Techasakul S. Antimicrobial activity of coronarin D and its synergistic potential with antibiotics. *Biomed Res. Int.*, 2014; **2**, 1–8.
35. Petric RC, Braicu C, Raduly L, Zanoaga O, Dragos N, Monroig P, Dumitrascu D, Berindan-Neagoe I. Phytochemicals modulate carcinogenic signaling pathways in breast and hormone-related cancers. Onco. Targets. Ther., 2015; **8**, 2053–66.
36. Aiyegoro O A, Okoh A. Use of bioactive plant products in combination with standard antibiotics : Implications in antimicrobial chemotherapy. 2009; J. Med. Plants Res., **3**, 1147–52.

Table No 01: Synergistic multi-target effects

Drug A & its mechanism of action	Drug B & its mechanism of action	Reported effects	Possible mechanism of action
Amoxicillin (inhibits bacterial cell-wall synthesis)	Clavulanate (β-lactamase inhibitor	Antibacterial synergy due to more effective drug distribution or localization	The presence of clavulanate improve the level of amoxicillin at bacterial cell wall by inhibiting its degradation enzyme β-lactamase
Sulphamethoxazole (DHPs inhibitor)	Trimethoprim (DHFR inhibitor	No synergism is reported. The only one after other individual effects reflects in the response	Sulphamethoxazole targets the upstream DHPs whereas, trimethoprim targets the downstream DHFR. Trimethoprim act as a backup when the effect of sulphamethoxazolee becomes less effective
Erythromycin (inhibit bacterial protein synthesis)	Penicillin (act on bacterial cell wall)	The combination shows both synergic and additive action due to facilitating action	Presence of penicillin boosts erythromycin penetration into bacterial cells, thereby improve its bioavailability

Table No: 02. Synergism due to Pharmacodynamics interaction

Drug A & its mechanism of action	Drug B & its mechanism of action	Reported effects	Possible mechanism of action
Cycloserine (Inhibits bacterial cell-wall synthesis)	Epigallocatechin gallate (interruption of bacterial cell wall integrity)	Destruction of bacteria cell wall due to the synergistic antibacterial action	The presence of both the component together complements each other. Epigallocatechin disrupts the integrity of bacterial cell wall and cycloserine act as an inhibitor for cell wall synthesis, which hampers the restoration of the cell wall.
Ampicillin (Interrupt bacterial cell-wall synthesis by blocking PBP2A	Daptomycin (break down of bacterial Cell membrane structure)	Strong synergistic antibacterial action	Membrane disruption due to daptomycin, perhaps supported by the presence of ampicillin
Artemisinin (Disrupts parasite mitochondrial function, modulates host immune function)	Curcumin (Generates ROs and produce cytotoxicity for malaria parasites)	Synergic/ Additive Antimalarial activities	They act at different sites in a non-interfering manner
Ampicillin (inhibit bacterial cell-wall synthesis by blocking PBP2A	Imipenem inhibits bacterial cell-wall synthesis by blocking PBP1A, 1B	Synergic / Additive antibacterial effect	Both act at the same active site. Due to the presence of both at relatively high MICs, May make it responsible for the better antibacterial effect

Table No 03: List of Some Bioactive Phytochemicals & their Role in Combination

Sl no	Combination of active molecules	Observed effect	Mechanism	Ref
1	Curcumin with Amphotericin B	Antifungal & antibacterial action.	A synergistically improve action was observed. In this case, the enzyme inhibitory effect of curcumin mainly reported being responsible for enhanced action.	[16]
2	Garlic Oil and Allyl Alcohol Derived from Garlic	Potent antifungal and anti-yeast effect	An additive action was observed. Garlic oil was reported to have cell damage capacity which facilitates the action of allyl alcohol, to show potential killing effect by affecting cytosolic components,	[17]
3	Garlic extract with ciprofloxacin	Antibacterial action	The presence of garlic extract improves the inhibitory action of ciprofloxacin.	(14)
4	Rifampicin with Nalidixic acid	Anti-microbial action	The combination showed enhanced antimicrobial action may be due to synergistic action. Path of mechanism not reported.	[18]
5	Fluconazole with Cardamom oil & Boswellia oil	Antifungal activity	Both the combination shows remarkable improved antifungal action may be due to synergism.	[19]
6	Curcumin with fluconazole	Antifungal action against fluconazole-resistant pathogens	The curcumin modulates MDR by inhibiting the transport of fluorescent substrates that are actively effluxes from cells. It improves the sensitivity of fluconazole and, at the same time, practically abolishing cellular growth.	[20]
7	Allicin with ketoconazole	Antifungal activity	The combination demonstrates potential antifungal activity due to synergism	[21]
8	Thymol with itraconazole (ITR) & fluconazole(FLU)	Potent antifungal action against resistant strain	Thymol enhances the action probably by disruption of the cell wall /membrane integrity mitogen-activated protein kinase (MAPK) system when used in combination with ITR. It probably creates lesions in the plasma membrane and disruption of ergosterol biosynthesis when used with FLU	[22]
9	Benzoic acid and its derivatives with fluconazole and itraconazole	Enhance antifungal action against resistant strain	Most effectively enhance the antifungal action of azole derivatives utilizing targeting of an oxidative stress response system	[22]
10	Caspofung in with ferulic acid	Antifungal action	The combination shows antifungal action due to synergism	[23]
11	Sulfamethoxazole with Myricetin	Synergistic antimicrobial action	The potency of combination increases due to synergism. Myricetin act by DNA binding and induce enzymatic DNA breakage	[24]
12	Tetracycline with epigallocatechin gallate	Synergistic antimicrobial action	Enhances the activity tetracycline against resistant staphylococcal by impairment of tetracycline efflux pump activity and increased intracellular retention of the drug	[25]
13	sulfamethoxazole with proto catechuic acid, Ellagic acid, and Gallic acid	Synergistic antibacterial and antifungal action	Improve activity may be due to DNA gyrase and topoisomerase IV enzymes	[24]

14	Sulfadiazine with Proto catechuic acid and Quercetin	Wide range of antimicrobial action	Reported to have synergism, however, exact mechanisms are unknown.	[26]
15	Kaempferol with Norfloxacin and Ciprofloxacin	Potent antibacterial action	Combinations show synergism and reported possible mechanism involved may be DNA gyrase and DNA topoisomerase IV	[27]
16	Ceftazidime with Quercetin analogs	Potent antimicrobial action	A possible reported mechanism states that cell wall damage due to leakage of potassium. Both the compound act at the same target either at different sites	[27]
17	Allium oils with ketoconazole	Fungi static activity	Reported to have synergism. The molecular mechanism was not reported	[21]
18	Berberine with 5'-Methoxyhydnocarpin	Potent antimicrobial action	5'-methoxyhydnocarpin (1mg/ml) inhibited the berberine effluxing multidrug pump and thus increased berberine bioavailability. When combined with subinhibitory amounts of berberine, 5'-methoxyhydnocarpin caused complete inhibition of growth at a concentration of 1 mg/ml.	[4]
19	Curcumin with 5-fluorouracil	Anticancer activity	Enhance the capacity of 5-fluorouracil due to synergism. Molecular path not reported.	
20	Quercetin with doxorubicin	Anticancer activity	Quercetin combined with cisplatin, exhibited a proapoptotic effect toward human laryngeal carcinoma cells	[28]
21	Resveratrol with Doxorubicin	Anticancer activity	Resveratrol facilitates doxorubicin uptake by the cells, probably by downregulation of the expression of *mrp-1* (*mrp-1* belongs to ATP-binding cassette transporter family, involved in multidrug resistance). It acts as an energy-dependent efflux pump whose overexpression causes a decrease in doxorubicin concentration in the cells	[29]
22	Silibinin with Aminoglycosides	Antibacterial action	Improve the action efficacy of aminoglycosides through a significant inhibitory effect on DNA topoisomerase activity due to the formation of complexes that alter enzyme binding	[30]
23	Kaurenoic Acid Derivatives with Fluconazole	Enhance antifungal action against the fluconazole-resistant strain	Kaurenoic Acid Derivatives enhance the capacity of fluconazole probably due to inhibition of topoisomerase I.	[31]
24	Glabridin combination with fluconazole	Effectively improve antifungal activity (fungicidal) of fluconazole	Glabridin facilities membrane permeability & damage cell wall, hence increase the performance of fluconazole.	[32]
25	lactoferrin with fluconazole &itraconazole	Synergistic Antifungal activity	Lactoferrin shows synergistic activity in combination with azole derivatives. A possible mechanism may be by promotion or suppression of ergosterol synthesis in the Candida cell membrane. Again the iron-chelating function of lactoferrin reported contributing in the synergism	[33]

26	Nisin with Thymol	Synergistic antimicrobial activity	Destabilization of bacterial membrane structure resulting in an increased permeability for nisin which leads to bacterial cell lysis.	[34]
27	Carnosic acid with Tetracycline	Antimicrobial activity	Possible mechanism reported inhibiting the MDR pumps	[3]

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REVIEW ARTICLE

Phytochemical and Pharmacological Overview of *Bombax ceiba* Flowers

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ABSTRACT

Bombax ceiba is a medicinal tree, referred to as the red silk cotton tree, and belongs to the family of Bombacaceae. This plant has traditional and ethnomedicinal uses for treating a variety of diseases. Flavonoids, terpenoids, saponins, phenolic compounds, tannins, glycosides, and carbohydrates are the active principles of the *Bombax ceiba* flower. Hepatoprotective, antioxidant, antiproliferative, cardioprotective, antiviral, antidiabetic, antitumor, analgesic, anti-inflammatory, and cytotoxicity protection are pharmacology. This article aims to discuss the phytochemicals and their several pharmacological activity studies of the *Bombax ceiba* flower.

Keywords: *Bombax ceiba*, flower, pharmacological, traditional.

INTRODUCTION

The family of Bombacaceae includes *Bombax ceiba*, a medicinal plant in tropical and subtropical India [1]. Red Silk cotton tree, Simul, Simbal, Indian kapok, Indian Bombax, Katsavar are some of its common names [2]. It grows in India's dry, moist, and mixed deciduous forests [3]. It is a massive and long-lived tree [4]. The leaves of this tropical tree are deciduous in the winter and have a straight, tall trunk. In spring, the five-petaled red blossoms develop when the tree does not have any new leaves. Flowering produces a capsule that includes white cotton-like fibres when fully ripe. To combat animal assaults, it has spikes on its trunk [5]. The flowers, which bloom from January to March on the leafless trees, are noticed. In winter and spring, when the naked branches are covered with enormous, meaty crimson blossoms, creating a stunning sight. Birds are drawn to them and are most likely the ones who

pollinate them. After dropping, these flowers leave a scarlet carpet on the ground for a few weeks (2-3 weeks). Semal's flowers are exceptionally showy, appealing, and visible from a considerable distance. People like to plant it as an ornamental plant in the botanical garden or as an avenue species because of its gorgeous and lovely blossoms. The flowers are numerous and enormous, measuring 10-12.5 cm in diameter. Towards the time of flowering, it gathers at the ends of the branches. Sepals are thick, meaty, and cup-shaped. Flower petals are 7.5-15 cm long, rectangular, recurved above, juicy, and vivid crimson, occasionally yellow or orange[6].For natives of several Asian countries, *Bombax ceiba* provides food, fodder, fibre, fuel, medicine, and various other items[7]. Phenolic chemicals (quercetin, kaemferol, Shamim, mangiferin, and naphthalene derivatives), glycosides, alkaloids, tannins, triterpenoids (sitosterol and lupeol), phytosterols, and proteins were discovered in the *Bombax ceiba*plant [8].These compounds are antioxidant, anti-inflammatory, immunomodulatory, hypotensive, hypolipidemic, cytotoxic, antineoplastic, and antihyperglycemic in nature[9].

Habitat and Distribution

It's a common tree in mixed deciduous forests, and it's a distinctive tree in grassy savannah plains, where it can quickly spread. Its widespread distribution is owing to the cotton-covered seeds being transported long distances by the wind[10]. Temperate Asia, tropical Asia, Africa, and Australia are all home to this species. It founds in India at altitudes of up to 1500 meters. It thrives on well-drained soils or deep sandy loams, especially on low land lying between hills, with an annual rainfall of 50 to 460 cm evenly spread throughout the year [11].

Scientific classification

Kingdom: Plantae
Division : Magnioliophyta
Class : Magnoliopsida
Order : Malvales
Family : Bombacaceae
Genus : Bombax
Species : Ceiba [12].

Morphological characteristics

Semal is a tall, deciduous tree with horizontal, upright branches, and the trunk bears numerous conical spines when it is young. Bark-It has a silver-grey or grey-brown bark with a stiff and pointed outgrowth. Leaves -Glabrous, spreading, large leaves with 5-7 lanceolate leaflets and smooth edges. Flowers -The tree is leafless when numerous red flowers bloom, and the stamens are organized into five bundles with 9-12 stamens in each bundle. Fruits -Dry fruits provide capsules that are 15 mm in length and which are loaded with numerous black seeds. Seeds-The form of seeds is obovoid and irregular with silky hair that makes them smooth. And these are encased in long white wool. Gum -It ranges in colour from light brown to dark brown, referred to as Semul gum [13].

Traditional uses

Barks have mild astringent, demulcent, diuretic, anti-inflammatory, tonic, and demulcent properties. It can also be used to treat freckles, acne vulgaris, and other skin and pigmentation issues on the face [14]. *Bombax ceiba* leaves are utilized in a variety of treatments for anaemia and sexual debility. Flowers are used in the treatment of cancer and paralysis [15]. Wounds, diarrhoea, and dysentery are treated with the plant's roots, while the gum is used to cure burning sensations, pulmonary TB, enteritis, and influenza. Fruits can help with chronic inflammation, kidney and bladder ulcers. Seeds are used to treat gonorrhea [16].

Ethnomedicinal uses

To treat mumps, the sensitive twig was used as a toothbrush. Menorrhagia was treated with powdered flowers combined with honey. For getting rid of acne, the thorn was taken and rubbed raw milk on stone, ground to make a

paste, and applied to the face as an ointment for 5–6 days. For treating mouth sores, Cordia gharaf stem barks were chewed with a crushed thorn. As a tonic, root bark extract was used to treat sexual debility and a nerve tonic to benefit nerves. To avoid Erectile Dysfunction, combine root powder with milk and sugar-sweetened condensed milk [17].

Phytochemicals

Flavonoids, terpenoids, tannins, phenolic compounds, saponins, carbohydrates, and glycosides are among the phytoconstituents found in *Bombax ceiba* flower extract [18]. Glycosides-quercetagetin and quercetagetin -3-O-D-glucofuranoside[19], cholesterol, stigmasterol, campesterol, α–amyrin, apigenin, linarin, xanthomicrol, cosmetin, and saponarin have been discovered in flowers[20]. Flavonoids found in *Bombax ceiba* flowers include rutin, quercetin, kaempferol-3-O-□-D-glucuronopyranoside, kaempferol-3-O-rutinoside, quercetin-3-O-D-glucuronopyranoside, quercetin-3-O-D-glucopyranoside, vicenin 2, sexangularetin-3-O-sophoroside, isovitexin, and vitexin, xanthones: 7-O-methyl mangiferin, mangiferin, isomangiferin, coumarins: fraxetin, scopoletin, esculetin and scopoletin, and other chemicals like benzyl-□ -D-glucopyranoside, blumenol C glucopyranoside, phenylethyl rutinoside, methyl chlorogenate, chlorogenic acid, protocatechuic acid, and vanillic acid[21]. Kaempferol, free □-setosterol, □-D-glucoside of □-setosterol, hentriacontanol, hentriacontane, and a trace of essential oil were found in methanol and n-hexane extracts of flowers. The flower's dried stamens yielded a polysaccharide containing L- rhamnose, L-arabinose, and D-galactose at a molecular ratio of 3:4:5. Two anthocyanidin glycosides identified in fresh flower petals are cyanidin-7 methyl-ether-3p-glucopyranoside and pelargonidin-5p-D-glucopyranoside [22].
The structure of some chemical compounds is given below.

Pharmacology

Hepatoprotective Efficacy

Ravi et al. investigated the hepatoprotective efficacy of a methanolic extract of *Bombax ceiba* flowers against hepatotoxicity caused by Rifampicin and Isoniazid in rats. Flower extract was given intraperitoneally in three doses (150, 300, and 450 mg/kg). Total bilirubin, aspartate transaminases (AST), alanine transaminases (ALT), and alkaline phosphatase levels all dropped significantly. However, they noticed an elevation in total protein when compared to the control group. They observed the flower extract increased the level of reduced glutathione and considerably reduced the amount of thiobarbituric acid- reactive compounds at all doses compared to the control. The researchers determined that extract might mitigate the effect of Rifampicin and Isoniazid to the point of necrosis but not reverse hepatic harm [23].*Bombax ceiba* flower aqueous extract (BCFAE) was examined for its hepatoprotective and antioxidant activities by Manish M. Wanjari. Carbon tetrachloride (CCl4) was used to cause hepatotoxicity in rats, and at the same time, Silymarin (25 mg/kg) or BCFAE (250 or 500 mg/kg) and vehicle were orally given for seven days. Severe impairment in liver function resulted from the delivery of CCl4, as evidenced by a considerable elevation of marker enzymes in plasma levels and histological liver damage on a large scale. *Bombax ceiba* flower aqueous extract therapy improved liver functioning deficits induced by CCl4, except for albumin and total protein. BCFAE therapy reduced total protein levels and avoided elevations in transaminase, glutamate, oxaloacetate, bilirubin, alkaline phosphatase, glutamic pyruvic transaminase and triglycerides induced by CCl4[24].

Cardioprotective activity

Based on biochemical and histological characteristics, Sita Sharan Patel et al. investigated the cardioprotective potential of *Bombax ceiba L.*, Malvaceae flower aqueous extract against myocardial infarction induced by Adriamycin (Adr) in rats and compared to vitamin E. For four weeks, *Bombax ceiba* extract was given orally at various doses (150,300 and 450 mg/kg, b.wt.) to Wister rats at a rate of six days a week. Adriamycin significantly (p0.001) reduced the SGOT and LDH level in the cardiac homogenate. A significant (p0.001) elevation in LDH in the cardiac homogenate and a reduction in LDH and SGOT in the vitamin E and Bombax ceiba treated groups. In Adriamycin-treated rats, microscopic examinations revealed myofibrillar loss, lipid inclusions, leukocyte infiltration, and mitochondrial enlargement. However, prior therapy of Bombax ceiba and vitamin E resulted in a decreased degree

of histological abnormalities. The findings imply that *Bombax ceiba* aqueous flower extract protects against cardiotoxicity induced by Adr and could be used for cardioprotective efficacy [25].

Analgesic, Anti-inflammatory, hepatoprotective and antitumor activities

(Said et al.) evaluated the methanolic extract of *Bombax ceiba* flowers for analgesic and anti-inflammatory, hepatoprotective and antitumor activities. They performed a toxicity test in mice; 70% of the methanolic extract (up to 5gm body weight) had no clear harmful impact. Flower extract of *Bombax ceiba L*. was investigated using the writhing test and the hot plate method for analgesic effects. After administering a 70 percent methanolic extract of *Bombax ceiba* flowers (25 and 50 mg/100 g.b.wt. The mean reaction time on the hot plate was significantly delayed, with percentage changes of 56.5 and 66.2 percent after 1 hour and 72.6 and 83.1 percent after 2 hours. Tramadol exhibited a considerable delay, with percent changes of 48 and 68 percent after 1 and 2 hours, showing systemic analgesic effects. Acetic acid-induced writhing was dramatically reduced in mice given two dose levels of the studied extract. The antinociceptive activity of the *Bombax ceiba* flowers 70 percent methanolic extract was 27.3 and 47.6%, with the highest reduction in writhing score, indicating a peripheral analgesic effect. A reduction in the reactivity to acute paw oedema was seen, indicating an anti-inflammatory effect. The decrease in the ALT and AST levels revealed the protective efficacy of the extract against liver damage induced by paracetamol. The activation of early antigen (percentage of Epstein-Barr virus early antigen-positive cells) inhibited by extract using an in-vitro EBV-EA activation test. The researchers concluded that *Bombax ceiba* flower extract exhibited analgesic, anti-inflammatory activities, hepatoprotective and antitumor activities [26].

Antioxidant and Antiproliferative Activity

Tiago O. Vieira and colleagues investigated several antioxidant assays were used to assess the antioxidant activity of *Bombax ceiba* flower, including (a) activity against peroxynitrite and ascorbyl free radicals that cause lipid peroxidation (in microsomes of rat liver and soybean phosphatidylcholine liposomes); (b)Hydroxyl free radicals and DPPH scavenging activity (1,1-diphenyl-2-picryl-hydrazyl); and (c) effect on myeloperoxidase activity. The results indicated that the *Bombax ceiba* flower methanolic extract showed antioxidant ability against ROS, and RNS was substantial [27]. Rosa Tundisa et al. tested the antioxidant ability and antiproliferative efficacy of *Bombax ceiba* flowers against seven cancer cell lines from humans. The antiproliferative effects of light petroleum and diethyl ether extracts were compared to a human standard cell line, 142BR, against ACHN, A375, C32, MCF-7, COR-L23, HeLa, and Lymph Node Carcinoma of the Prostate (LNCaP) cells. In human kidney adenocarcinoma (ACHN), both light petroleum and diethyl ether extracts showed the most substantial antiproliferative impact. Flower extracts also demonstrate antioxidant activity. The antioxidant activities of the sections were studied using GC-MS (gas chromatography-mass spectrometry) and in vitro systems such as the carotene bleaching test, Fe-chelating activity, and the DPPH assay. The diethyl ether extract exhibited more antioxidant activity in the -carotene bleaching test, and the light petroleum extract was the most active in the DPPH radical scavenging test [28].

Antioxidant and Antiviral activity

Yu-Bo Zhang et al. used the CPE and plaque reduction assay to assess the antiviral activity against RSV (respiratory syncytial virus). Antioxidant properties were determined using (DPPH) radical-scavenging assays and the Ferric-reducing antioxidant power (FRAP) assay. Flower extracts contain chemicals such as kaempferol-3-O-O-E-p-coumaroyl)-D-glucopyranoside, quercetin, and mangiferin possess antiviral properties. The Kaempferol-3-O-(6"-O-E-p-coumaroyl)-D-glucopyranoside showed the highest anti-RSV action comparable to the positive medication ribavirin. According to the findings, the *Bombax malabaricum* flower indicated the presence of antioxidant and antiviral effects [29].

Cytotoxicity Protection

In HT1080 cells, Souichi Nakashima et al. discovered protective properties against BaP-induced cytotoxicity. The flower extract yielded 16 compounds, including mangiferin, two ascorbic acid derivatives, and four butyrolactones.

Compounds like kaempferol 3-O-D glucopyranoside, quercetin 3-O-D glucopyranoside, two flavonoids (-)-loliolide, and butyrolactone derivative, protect against cytotoxicity. One of the active components, quercetin, had a more negligible effect on the aglycone than the glycoside. *Bombax Ceiba* and its components have a protective effect against BaP-induced cytotoxicity [30].

Antibacterial and Antioxidant Activity

Antibacterial susceptibility, antioxidant testing, and phytochemical screening were performed by Deepshikha Rathore et al. In the methanolic extract; phytochemical screening showed the flowers of *Bombax ceiba* are high in alkaloids, amino acids, tannins, and phenolics. Using the DPPH technique, the antioxidant ability was determined. More than 90percent antioxidant efficacy was observed in semi-purified extracts. They had performed an antibacterial activity zone of inhibition test and established the sensitivity level. The methanol extract of the flower was effective against both gram-positive (*Bacillus subtilis, Staphylococcus aureus I* and*II*) and gram-negative bacteria (*Pseudomonas aeruginosa Escherichia coli and Klebsiella pneumonia*). The fact that gram-positive bacteria are enclosed by the cell wall or a thick peptidoglycan layer could explain why they are active against them. This has a low resistance to small molecule diffusion. The chemicals described here could easily pass through the gram-positive bacteria's porous outer shell. Gram-negative bacteria's pathogenic potential is usually linked to specific components of their cell walls, particularly the lipopolysaccharide layer (LPS). They have channels of tiny pores through which newly generated semi-synthetic compounds diffuse freely and exhibit antibacterial action. The increased activity of each molecule could be attributed to increased cellular absorption and hence increased drug bioavailability. The broth dilution test was used to obtain the MIC values of the extract. The antibacterial activity of MIC ranged from 3.125 to 12.500 g/mL in the extract. The findings showed that *Bombax ceiba* flower extract could be used as an antibacterial agent. They have channels of tiny pores through which newly generated semi-synthetic compounds diffuse freely and exhibit antibacterial action. The increased activity of each molecule could be attributed to increased cellular absorption and hence increased drug bioavailability. The broth dilution test was used to obtain the MIC values of the extract. The antibacterial activity of MIC ranged from 3.125 to 12.500 g/mL in the extract. The findings showed that *Bombax ceiba* flower extract could be used as an antibacterial agent [31].

Antioxidant and Antidiabetic Activity

(Kriintong et al.) investigated the antioxidant and antidiabetic activities of *Bombax ceiba.* The 95 percent ethanol floral extract had the strongest glucosidase and amylase inhibitory activity. The 95 percent ethanol floral extract had the maximum antioxidant property in the ferric reducing antioxidant power (FRAP) experiment. The crude extracts of *Bombax ceiba*, especially the floral extracts, demonstrated substantial antioxidant and antidiabetic effects *in vitro* [32].

CONCLUSION

This article has outlined the phytoconstituents and pharmacological properties of the *Bombax ceiba* flower. It is a traditionally well-accepted plant used to treat the various physiological condition. The *flavonoids, terpenoids, saponin, phenolic compoun*ds, tannins, glycoside, and carbohydrates are present in *the Bombax ceiba flower.* These phytoconstituents showing hepatoprotective, antioxidant, antiproliferative, cardioprotective, antiviral, antidiabetic, analgesic, and anti-inflammatory activity. Hence, future research is required to investigate the chemical profile and pharmacological properties of *the Bombax ceiba* flower for treating various diseases.

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CONFLICT OF INTEREST

There are no conflict of interest.

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Nil

ABBREVIATIONS

EBV-EA: Epstein –Barr Virus Early Antigen; **LDH:** Lactate Dehydrogenase; **SGOT:** Serum Glutamic Oxaloacetic Transaminase; **ROS:** Reactive Oxygen Species; **RNS:** Reactive Nitrogen Species; **CPE:** Cytopathic Effect; **MCF-7:** Michigan Cancer Foundation-7; **COR-L23:** Human Caucasian lung large cell carcinoma; **HeLa:** Henrietta Lacks.

REFERENCES

1. Anandarajagopal, K., Sunilson, J. A. J., Ajaykumar, T. V, Ananth, R., & Kamal, S. (2013). *In-vitro Anti-Inflammatory Evaluation of Crude Bombax ceiba Extracts*. *3*(1), 99–104.
2. Ravi, C., & Yogesh, S. (2019). BOMBAX CEIBA LINN.: A REVIEW.
3. Bhavsar, C., & Talele, G. S. (2013). Potential anti-diabetic activity of *Bombax ceiba*. *Bangladesh journal of pharmacology*, *8*(2), 102-106.
4. Verma, S. K., Jain, V., & Katewa, S. S. (2011). Anabolic effect of *Bombax Ceiba* Linn. root in idiopathic involuntary weight loss–a case study. *Journal of Herbal Medicine and Toxicology*, *5*(1), 1-5.
5. Pankaj, H., Pallavi, D., Sharada, L., & Somshekhar, S. (2014). *Pharmacognostical and phytochemical studies on roots of Bombax ceiba Linn.*
6. Hossain, M. M. (2016). *Pharmacological investigations of roots of Bombax ceiba* (Doctoral dissertation, East West University).
7. Gao, Y., Wang, H., Liu, C., Chu, H., Dai, D., Song, S., Yu, L., Han, L., Fu, Y., Tian, B., & Tang, L. (2018). *De novo genome assembly of the red silk cotton tree (Bombax ceiba). May*, 1–7. https://doi.org/10.1093/gigascience/giy051
8. Gadge, N. B., & Jalalpure, S. S. (2012). *Fruit reduces the risk of calcium oxalate urolithiasis in rats. Curative treatment with extracts of Bombax ceiba fruit reduces the risk of calcium oxalate urolithiasis in rats*. *0209*. https://doi.org/10.3109/13880209.2011.604332
9. Wang, G. K., & Lin, B. Bin. (2012). *Natural Product Research : Formerly Natural Product Letters A new lignan with anti-HBV activity from the roots of Bombax ceiba*. *March 2013*, 37–41. https://doi.org/10.1080/14786419.2012.740032
10. Forest, T., Management, R., Willey, J., Delhi, N., & Characteristics, T. (2004). *Correlation and Path Analysis Studies Between Biomass and Other Characters in Bombax ceiba L .6*, 5–6.
11. Karole, S., Gautam, G., & Gupta, S. (2017). *PHARMACOGNOSTIC AND PHARMACOLOGICAL*. *6*(3).
12. Linn, B. (2019). *Review on Ethnobotany Phytoconstituents and Phytopharmacology of Bombax ceiba*. *March*. https://doi.org/10.21276/ijpbs.2019.9.1.136
13. Chaudhary, P. H., & Tawar, M. G. (2019). *Pharmacognostic and Phytopharmacological Overview on Bombax ceiba*. *10*(1), 20–25.
14. Zahan, R., Nahar, L., Haque, M., Nesa, M. L., & Alam, Z. (2013). *Antioxidant and Antidiabetic Activities of Alangium salvifolium and Bombax ceiba*. *12*(March 2011), 159–163.
15. Depani, P., Gadhvi, K., & Vyas, S. (2019). *Ethnobotanical Potential and PhytochemicalScreening of Bombax ceiba L .29*(4), 1–8. https://doi.org/10.9734/EJMP/2019/v29i430163
16. Sharma, N., Kispotta, S., & Mazumder, P. M. (2020). *Immunomodulatory and anticancer activity of Bombax ceiba Linn leaf extract*. *10*(April), 426–432. https://doi.org/10.4103/2221-1691.290134
17. Upadhyay, B., Dhaker, A. K., & Kumar, A. (2010). Ethnomedicinal and ethnopharmaco-statistical studies of Eastern. *Journal of Ethnopharmacology*, *129*(1), 64–86. https://doi.org/10.1016/j.jep.2010.02.026
18. Hait, M., & Goswami, J. (2017). *Physicochemical and phytochemical status on the flower of Bombax ceiba*. *5*(3), 189–192.
19. Kumar, N. S., & Madhurambal, G. (2010). Quercetagetin Glycoside from the Flowers of *Bombax ceiba*. *Asian Journal of Research in Chemistry*, *3*(1), 78-80.
20. El-hagrassi, A. M., Ali, M. M., & Osman, A. F. (2011). *Natural Product Research : Formerly Natural Product Letters Phytochemical investigation and biological studies of Bombax malabaricum flowers*. *March 2013*, 37–41.

21. Joshi, K. R., Devkota, H. P., & Yahara, S. (2013). *NPC Natural Product Communications*. *8*, 7–8. https://doi.org/10.1177/1934578X1300800508
22. Rani, S., Rahman, K., & Sultana, A. (2016). Ethnomedicinal and pharmacological activities of Mochrus (*Bombax ceiba* Linn.): An overview. *CELLMED*, *6*(1), 2-1.
23. Ravi V, Patel SS, Varma NK, Dutta D, Saleem TSM. Hepatoprotective activity of Bombax ceiba Linn against isoniazid and rifampicin-induced toxicity in experimental rats. IJARNP. 2010;3:19-26.
24. Wanjari, M. M., Gangoria, R., Dey, Y. N., & Gaidhani, S. N. (2016). *flowers against carbon tetrachloride-induced hepatotoxicity in rats*. https://doi.org/10.20517/2394-5079.2015.55
25. Patel, S. S., Verma, N. K., Rathore, B., Nayak, G., Singhai, A. K., & Singh, P. (2011). *flowers against acute adriamycin-induced myocardial infarction in rats*. *21*(4), 704–709. https://doi.org/10.1590/S0102-695X2011005000090
26. Said, A., Aboutabl, E. A., Nofal, S. M., Tokuda, H., & Raslan, M. (2011). Phytoconstituents and bioactivity evaluation of Bombax ceiba L. flowers. *Journal of Traditional Medicines*, *28*(2), 55–62. https://doi.org/10.11339/jtm.28.55
27. Vieira, T. O., Said, A., Aboutabl, E., Azzam, M., & Tânia, B. (2013). *Bombax ceiba Antioxidant activity of methanolic extract of Bombax ceiba*. *0002*. https://doi.org/10.1179/135100009X392485
28. Tundis, R., Rashed, K., Said, A., Menichini, F., &Loizzo, M. R. (2014). In vitro cancer cell growth inhibition and antioxidant activity of *Bombax ceiba* (Bombacaceae) flower extracts. *Natural product communications*, *9*(5), 1934578X1400900527.
29. Zhang, Y., Wu, P., Zhang, X., Xia, C., Li, G., & Ye, W. (2015). *Phenolic Compounds from the Flowers of Bombax malabaricum and Their Antioxidant and Antiviral Activities*. 19947–19957. https://doi.org/10.3390/molecules201119660.
30. Nakashima, S., Oda, Y., Ogawa, Y., Nakamura, S., Uno, M., Kishimoto, M., Yoshikawa, M., & Matsuda, H. (2018). *NPC Natural Product Communications*. 8–11. https://doi.org/10.1177/1934578X1801300512
31. Rathore, D., Srivastava, R., Geetanjali, G., & Singh, R. (2019). Phytochemical screening, antioxidant and antibacterial activities of *Bombax ceiba* flower. *ALGERIAN JOURNAL OF NATURAL PRODUCTS*, *7*(1), 651-656.
32. Kriintong, N., & Katisart, T. (2020). *In vitro, Antioxidant and Antidiabetic Activities of Leaf and Flower Extracts from Bombax ceiba*. 194–198. https://doi.org/10.4103/pr.pr

Fig 1: *Bombax ceiba* tree with flowers

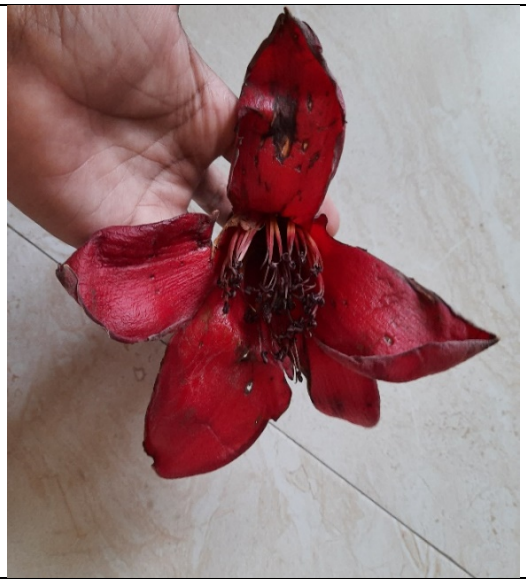
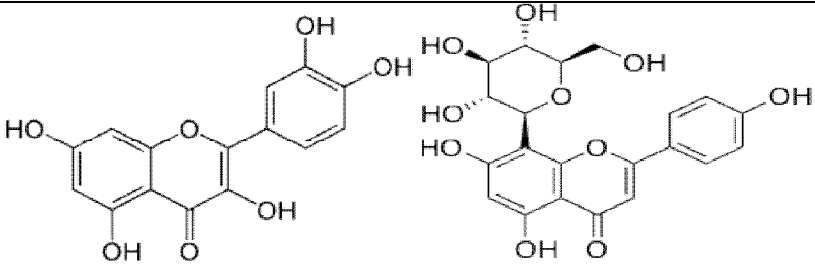
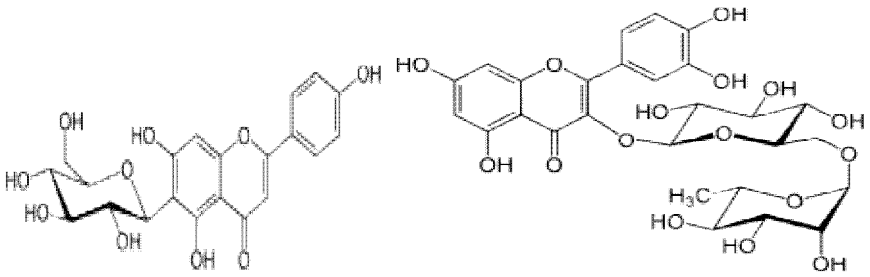


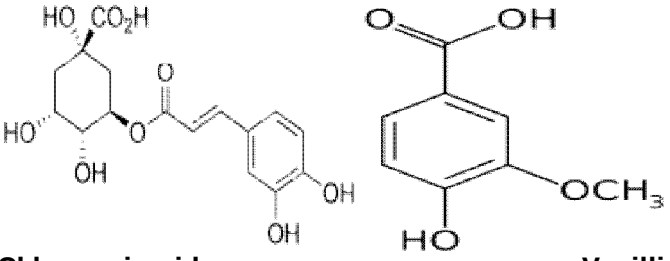
Fig 2: *Bombax ceiba* flower



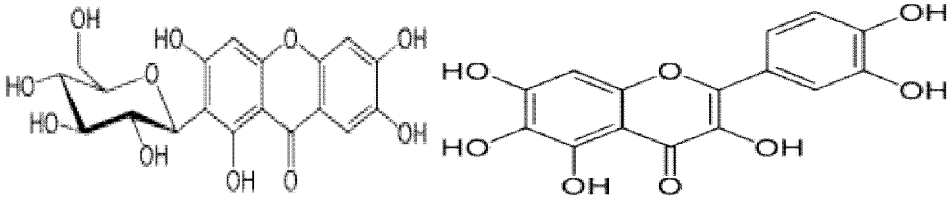
Quercetin **Vitexin**



Isovitexin **Rutin**



Chlorogenic acid **Vanillic acid**



Mangiferin **Quercetagetin**

Fig 3: Phytochemicals of *Bombax ceiba* flower

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RESEARCH ARTICLE

Accident Alert and Safety System for Differently-Abled and Elderly People using Machine Learning

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ABSTRACT

Differently-abled is an umbrella term that covers conditions, limits of movement, and restrictions on participation. Fifteen percent of the world's population encompasses the differently-abled community of a differentiated range and it is a vivid fact that sufficient attention is not paid to the differently-abled people and the elderly people, residing inside the residence, such as where there is no guardian available. They can face various types of accidents and situations that are helpless. Therefore, this research was carried out to produce a developed asset that supports the detection and generation of a signal during which the utmost care and attention are required. It sends an alert message about the situation to the guardian's mobile phone and provides a video inspection facility for the guardian using any web browser. The developed asset is conducted as an oriented assistive technology scenario that is supported by video and image processing with machine learning technology. Statistical analysis was revealed that the developed device can send an alert message to the guardian within23.60s at a 95% confidence level under 4G connection. Further, analysis proved that the system can play an important role in protecting human lives. The potential study in this respect is almost a success and improvements can be made by adding some advanced features such as sending alert messages to the health care provider emergency units.

Keywords: Differently-abled safety, elder safety, machine learning, image processing, signal generation.

INTRODUCTION

Disability can occur in the mind or body under any conditions and this makes it difficult for a person to involve with their activities and world interactions that are around them. Further, Disability can be categories under several types which are affected by peoples' vision, movement, thinking, remembering, learning, communicating, hearing, mental health, and social relationships[1]. According to the International Disability Foundation records, there are more than half a billion people are differently-abled all around the world and the count is increasing yearly[2] and some Statistics show that the global disabled population is between 235 million to 549 million[3] and according to the world bank records 15% of the world's population experience in some form of disability[4]. There are few types of disabilities, vision impairments, deaf or hard of hearing, mental health conditions, intellectual disability, acquired brain injury, autism spectrum disorder, and physical disorder [5]. A physical disability is permeant or substantially limits according to the physical ability or motor skills [6] and among all the types of disabilities, the highest rate of disabilities and major consideration was recorded in the type of physical disability.

In addition, aging causes disabilities due to various reasons. According to the United Nations (UN) world, population aging highlighted that there were 703 million people aged sixty-five years or over in the world in 2019. The aged population is increased by 9% in 2019 and is projected to rise further by 16% in 2050 according to the UN highlights. One out of ten persons over sixty-five years of age experiencing some difficulty in walking and for frail elderly 80% have difficulty or cannot walk at all [7]. Elderly and people with physical disabilities experiencing some kind of walking or mobility issues [8] [9]. People have to move for mobility aids and assistive technologies to perform their daily activities. Today, thousands of products based on assistive technology, are on the market to help empower people with mild to severe disabilities and with a wide range of individualized needs, from the simple to the most complex [10]. Assistive technology includes tremendous varieties, something like low tech as a cane to complexes like bionic limbs [10], wheelchairs, electronic communication systems, computer adaptations, and thousands of other applications [11].

Among all those mobility aids and assistive devices, most people are using wheelchairs as their assistive devices to assist their activities [12]. The number of wheel-chair users is increasing year by year [13] and it proves that people tend to use wheelchairs due to their disabilities. However, using a wheelchair leads to various kinds of accidents that affect a whole person's life. A person who relies on a wheelchair can be faced with an accident that causes fatal and non-fatal injuries [14]. They can experience adverse events such as falls that reduce their quality of life [15]. The accidents associated with wheelchairs and other assistive devices should be emphasized and prevention should be an integral part of the clinical and social practices[14]. It's a good quality of a society and a responsibility to treat differently-abled and elderly people with equality and equity. The sustainable development agenda in 2030 and sustainable development framework clearly state that considering differently-abled and elderly people with respect and as an important part is an agreeable characteristic in a developed society. It's very important to defend their human rights and taking care of their protection [16].

While treating mobility issues of elderly and differently-abled people by using assistive devices, society has to consider the side effects and factors like accidents that arise with those devices. Therefore, society has to be alert about that matter and there are lacking shreds of evidence to treat this issue. The researcher found that there are not enough safety systems to prevent accidents that can be happened while using assistive devices like wheelchairs and this research was conducted to achieve this matter and find a feasible solution using machine learning technology with image processing. In literature, researchers have combined image processing and assistive technology to build assistive devices in various ways. A mobile embedded system for human-computer communication in assistive technology has been developed using image processing technology and eye movement is detected by a special device and voluntary eye blinking is correlated with a pictogram to identify patient's needs [17]. Several technologies; such as a Global positioning system (GPS), a navigation system, and image processing technology,

were applied to develop devices based on navigation and orientation [18]. Further, these techniques were used in automated wheelchairs. In addition, some have tracked the headmotion to control the wheelchairs. Those are hands-free controller systems that detect head and neck movements to control the speed of the wheelchair and the direction [19][20][21]. The absolute number of people with mobility difficulties will grow over the next decades. Today, only 12.8% of U.S. residents are older than 65 years, but this will surpass 20% by 2030 with aging "baby boomers" [22]. It is emphasized that how the importance of mobility issues and elderly people in the research. However, minimum numbers studies were addressed to study these issues in the past. Moreover, there are limited studies to investigate and detect these issues using assistive devices.

This technique was adopted to solve several other problems. Nevertheless, studies do not focus on the combination of mobility issues with the assistive devices to cater to elderly people with the purpose of their safety. In the current competitive environment, people are busy with their day-to-day work life and they do not have time to look after their eldered family members, differently-abled members, or any family member with issues of mobility. Moreover, those elderly and differently-abled people with mobility issues are considered a burden for the country's economy. Therefore, by considering all the mentioned issues researcher found the niche area as a need of a solution for the people to take care and ensure the safety of their elderly family members and differently-abled or people having mobility issues in their families within their busy schedule'. As a solution for the gap, the researcher going to implement a system using machine learning and image processing to mount on assistive devices and it will ensure the safety of the elderly people and differently able people in dangerous situations while they have to stay alone. Because they do not need a guardian for the elderly people and they do not want to make extra effort or time for the ones who are in wheelchairs.

METHODOLOGY

Background

The purpose of this work is to implement an active and passive safety system to make sure the differently-abled and elderly person's safety when a guardian is not available within reach while developing a visual inspection method to observe the elderly/differently-abled persons. This application can be used by anyone with a Mobile phone operating system. Various Technologies and existing systems were provided a different type of help and broad analysis us to develop our new active and passive safety system for disabilities. Following technologies were utilized to develop the system more accurately and Figure 1 shows the construction of the system based on different technologies under hardware and software.

CAD software

CAD (Computer-aided drafting) or CADD (Computer-aided designing and drafting) is a technology that is used for design and technical documentation which replaces the traditional manual drafting method with an automated process [23].

Raspberry Pi

Raspberry Pi is a series of small single-board computers that is a good choice as low power, general purpose computer with low cost [24].

Open CV

Open CV (Open-source computer vision) is a library with a wide range of modules that helps with computer vision problems. It provides a framework to easily work with images and videos without worrying about the memory allocation and deal location for images [25].

Numpy

Numpy is a library with a fundamental package for scientific computing. It provides multidimensional array objects and an assortment of routines, including mathematical, logical, and random simulation and much more [26].

Haarcascade file

Cascade classifier is a machine learning-based approach trained with a lot of positive and negative images. This Haarcascade file or classifier can be used to identify objects in an image or a video [27].

Machine learning

Machine learning is an evolving method of computational algorithms that are designed to emulate human intelligence by learning from the surrounding environment [28]. It is a branch of Artificial intelligence and Computer science that focuses on the use of algorithms and data to imitate the way humans learn, gradually increasing its accuracy. The workflow of the system is shown in Figure 2 and it is explained in the following subsections.

Design of the model

Physical design and the architecture of the system were decided and modeled considering the selected hardware and the system expectations. The actual design of expected design was modeled with 3D modeling tools. Structure, all parts, and assemblies were designed using CAD software to get a proper idea of the design and it made the simulation and virtual inspection ease. Different factors were considered when designing the system as mountingcapability, height changing ability of the camera, the safety of the system, etc. (Figure 3 and Figure 4). The system was physically constructed using this CAD design and detailed drawings it made the construction error-free as shown in figure 5.

Hardware Selection

The success of this system depends on the processing capability of the processor as this system deals with many IO signals and for identification and classification of these signals and images needs good processing power. In an approach for the image processing and classification's ability of the processor and RAM capacity was highly expected. The ability to connect to the internet, Wi-Fi, and the ability to provide HDMI output was considered when selecting the development board. Considering all these factors Raspberry PI model B+ was selected as the microprocessor (Fig. 9.).Raspberry Pi Model B+ can manage and process all signals without getting jammed. It is very important to select a camera module that supports the Raspberry pi mini-computer and also has a good resolution incapable of image processing and video processing. When selecting the camera, the qualities listed below were considered (Fig.9).

- Maximal resolution under optimal condition
- Fine resolution or large inspection area
- Light sensitivity
- Dynamic range
- Signal-to-noise ratio
- Cost-effectiveness

Raspberry pi has a camera slot interface (CSI) for raspberry pi cameras and the Raspberry pi v1 camera was selected for this system. In this case, the dark and low contrast images taken using the Raspberry pi camera module are enhanced to define the image field. The Raspberry Pi 3 has a micro USB supply of + 5.1V. The exact amount of current (mA) provided by Raspberry Pi depends on what you are connecting to it. Model B is typically using 700-1000mA depending on which peripherals are connected. A power bank was used as a power supply for this system. The ability to recharge the power bank is made it easy to develop this system as it can be used for a long time without interruption.

Software selection

There are few operating systems for the Raspberry Pi microprocessors and Raspbian OS was selected as the OS for Raspberry PI model B+. Python was used as the programming language. Numpy and OPENCV were used as the libraries for programming. Haarcascade file was created using classification algorithms with machine learning technology(Appendix 1). To create Haarcascade file thousand of images were used to train the machine to identify the people and the situation. This Haarcascade file was used in this system to identify the people to make the decisions based on the situation to recognize accidents. Modeled assemblies were 3D printed and assembled and the camera was mounted in the camera mount and adjustability of the camera were considered when mounting. With python, raspberry pi was programmed and connected to the internet via WI-FI to send an alert message through the cloud database. This system always monitors the people and when the accident happened, this system analyzes the situation and sends an alert message regarding the accident to the guardian's WhatsApp account through the internet and guardian can inspect the patient at any instance with a video using any browser with given IP address(Appendix 1). The process of the system is shown in figure 6. This system was mounted with the wheelchair and tested the accuracy under different conditions. Mainly the effect of the internet signal strength and the lighting conditions were considered and the two trials with ten attempts were conducted under different internet speeds and diverse places to analyze the accuracy and the communication speed of the alert message to the guardian.

RESULTS AND DISCUSSION

This system was mounted on a wheelchair and tested the alert messages sending accuracy, receiving time (in seconds), and video feeding capabilities. Alert message sending function works well as expected and message receiving time can be varied with the internet speed. Alert messages sending time was measured under 4G and 3G connections under two trials in different situations. The results obtained and the average time taken to receive the message was summarized in Table 1 and the scatter plot of attempt concerning the time was depicted that how data are varied according to 4G and 3G connections. Figure 7 was indicated that two different identifications of times according to internet connections. It shows a wide spread of time range in the 3G connection than the 4G connection. This implies that the time taken to receive the message under 3G connection was high varied than 4G connections. It was further verified by the summary statistics in Table 2 and the interval plot of 95% confidence interval (CI) under two connections (Figure 9).

The average time taken to receive the message under a 4G connection is 23.60s (±0.47) and it was 30.35s (±0.92) in a 3G connection. Further, variation of the time taken for 3G connection is higher than the 4G connection due to relative high CV value in 3G connection. It can be interpreted that using this system, the guardian can receive the alert message within 23.60s when the differently-abled or elderly people have faced an accident if they have a powerful data connection. Additionally, its illustrations that the signal strength of the connection can affect the alert message sending time. If the internet connection has proper strength guardian can receive the message within few seconds. Correspondingly, this system waits 10 seconds waiting time to get clear idea about the situation. However, the internet strength and speed are affecting the video inspection and it requires a 4G LTE connection to take video footage.

Identification of human beings has been done using image processing technology. Detection of human beings is depending on camera capabilities. This system works well in the day time. However, in the night-time, it was not much accurate because of the low light. The camera used can capture human beings and actions accurately when the light intensity is high. Because the raspberry camera was failed to access the night vision ability. Therefore, this problem can be solved by using a proper night vision camera easily. In addition, the camera used for this system can't capture or record the sound. It's better to use a camera that can capture the sounds. Thus, the guardian can have a better idea about the accident and also guardian can communicate properly with the differently-abled or elder person directly.

CONCLUSION AND FUTURE DIRECTIONS

Disability can be a physical disability or a mental disability. The main category of disability is a physical disability and major concern is gain by physical disabilities. There are a higher number of elderly people in the world and most of them are experiencing some kind of disability with their age. For people with disabilities need assistance to perform their activities in day-to-day life and assistive technology is developed to assist them. There are plenty of assistive devices in the world market but they also had issues for patients. Such as several accidents while using those devices. And also, there should be a guardian to nursing them. For considering the above reasons researchers were developed the idea and the mechanism to solve the problem and ensure the safety of differently-abled and elderly people. It was an active and passive safety system for differently-abled and elderly people. Test runs indicate the positive results and overall expected results are arisen by using this system. Therefore, this system can use the health care industry as a less time-consuming system for the differently able people in the world within this competitive environment and busy lifestyles.

This system can identify the existence of a human being. The Haarcascade file was formed to detect humans and their actions. As a future direction, a facial expression recognition system can be cooperated, identify the person and his/her disability condition. Such features can make each person unique and non-identical in the system. The disability condition can be monitored by developing a facial expression detection system. Inspection of the accident through the video by the guardian is working correctly. This system has a one-way video communication ability. As further development, this can develop to achieve two-way communication. For that, it's necessary to connect a display with the raspberry pi. Raspberry Foundation has introduced the display with HD quality. Then differently-abled or elder persons can communicate with their guardian using the system connected to the wheelchair.

REFERENCES

1. Centers for Disease Control and Prevention. 2020. Disability and Health Overview | CDC, https://www.cdc.gov/ncbddd/disabilityandhealth/disability.html, [Accessed 5 December 2020].
2. Titchkosky, "*Disability, Self, And Society",* University of Toronto press., (2003). pp. 6-7.
3. R. L. Metts, Disability issues, trends, and recommendations for the World Bank (full text and annexes), Washington, World Bank, (2000).
4. World Bank. 2020. Disability Inclusion Overview. [online] Available at: <https://www.worldbank.org/en/topic/disability#:~:text=Results-,One%20billion%20people% 2C%20or%2015%25%20of%20the%20world's %20population% 2C,million%20people%2C%20experience%20significant%20disabilities.> [Accessed 5 December 2020].
5. Services.anu.edu.au. 2020. Different Types Of Disabilities - Staff Services - ANU. [online] Available at: <https://services.anu.edu.au/human-resources/respect-inclusion/different-types-of-disabilities> [Accessed 9 December 2020].
6. N. Bianquin, D. Bulgarelli, S. Besio, and V.Stancheva-Popkostadinova, "Conceptual Review of Disabilities", *Play development in children with disabilities.*, vol.3,(2017),pp.71-87.
7. E. Williams, Specialist Equipment Services for Differently abled People. The Need for Change. Royal College of Physicians, London, (2004).
8. P. Clarke, J. A. Ailshire, M. Bader, J. D. Morenoff, and J.S. House, "Mobility disability and the urban built environment", *American journal of epidemiology*., vol. 168, no. 5, (2008), pp. 506-513.
9. S. Pakjouei, A. Aryankhesal, M. Kamali, and S. H. Seyedin, "Experience of people with physical disability: mobility needs during earthquakes", Journal of education and health promotion., vol. 7, (2018).
10. M. K. Dove, "Advancements in assistive technology and AT laws for the disabled", *Delta Kappa Gamma Bulletin.*, vol. *78, no.* 4, (2012), p.23.
11. R. Sharma, and S. G. Wasson, "Speech recognition and synthesis tool: assistive technology for physically disabled persons", International Journal of Computer Science and Telecommunications., vol. 3 no. 4, (2012) pp. 86-91.

12. M. P. LaPlante, and D. S. Center, "Demographics of wheeled mobility device users", In *Conference on space requirements for wheeled mobility*. Center for Inclusive Design and Environmental Access, Buffalo, New York, (2003) October.
13. S. Kaye, T. Kang, and M. P. LaPlante, "Mobility device use in the United States", National Institute on Disability and Rehabilitation Research, US Department of Education.,vol. 14, (2000).
14. W. Y. Chen, Y. Jang, J.D. Wang, W. N. Huang, C. C. Chang, H. F. Mao, and Y. H. Wang, "Wheelchair-related accidents: relationship with wheelchair-using behavior in active community wheelchair users", *Archives of physical medicine and rehabilitation.*, vol. *92, no.* 6, (2018), pp. 892-898.
15. S. E. Slaughter, A. S. Wagg, C. A. Jones, D. Schopflocher, C. Ickert, E. Bampton, A Jantz, D. Milke, C. Schalm, C. Lycar, C. A. and Estabrooks, "Mobility of vulnerable elders study: Effect of the sit-to-stand activity on mobility, function, and quality of life", *Journal of the American Medical Directors Association.*,vol. *16, no.* 2, (2015), pp. 138-143.
16. I.Segovia-San-Juan, I. Saavedra and V. Fernández-de-Tejada, "Analyzing disability in socially responsible companies", Social Indicators Research., vol. 130, no. 2, (2017), pp.617-645.
17. R. G. Lupu, F. Ungureanu, and R. G. Bozomitu, "Mobile embedded system for human computer communication in assistive technology", In 2012 IEEE 8th International Conference on Intelligent Computer Communication and Processing,IEEE., (2012), pp. 209-212.
18. K. Patel and B. Parmar, "Assistive device using computer vision and image processing for visually impaired; review and current status", Disability and Rehabilitation: Assistive Technology., (2020), pp. 1-8.
19. A, Pajkanović, and B. Dokić, "Wheelchair control by head motion", *Serbian Journal of electrical engineering.*, vol. *10, no.* 1, (2013), pp. 135-151.
20. D. J. Kupetz, S. A. Wentzell, and B. F. BuSha, "Head motion controlled power wheelchair", In Proceedings of the 2010 IEEE 36th Annual Northeast Bioengineering Conference (NEBEC),IEEE., (2010), pp. 1-2.
21. A. Kondori, S. Yousefi, L. Liu. and H. Li, "Head operated electric wheelchair", In 2014 Southwest Symposium on Image Analysis and Interpretation,IEEE., (2014), pp. 53-56.
22. L. I. Iezzoni, E. P. McCarthy, R. B. Davis, and H. Siebens, "Mobility difficulties are not only a problem of old age", Journal of general internal medicine., vol. 16, no. 4,(2001), pp. 235-243.
23. Study.com. 2021. [online] Available at: <https://study.com/academy/lesson/what-is-cad-software-definition-uses.html> [Accessed 3 January 2021].
24. E. Upton and G. Halfacree, "Raspberry Pi user guide", John Wiley & Sons., (2014).
25. S. Brahmbhatt, *Practical OpenCV*, Apress, (2013).
26. Numpy.org. 2021. What is NumPy? — NumPy v1.20 Manual. [online] Available at: <https://numpy.org/doc/stable/user/whatisnumpy.html> [Accessed 4 January 2021].
27. Medium. 2021. Haar Cascades, Explained. [online] Available at: <https://medium.com/analytics-vidhya/haar-cascades-explained-38210e57970d> [Accessed 3 January 2021].
28. El Naqa, and M. J. Murphy, "What is machine learning?. In machine learning in radiation oncology", Springer,Cham, (2015), pp. 3-11

Table 1. Time (waiting time + sending time) Taken to Receive the Alert Message (in seconds)

Attempt	3G Connection			4G connection		
	Trial 1	Trial 2	Average	Trial 1	Trial 2	Average
1	25.0	45.0	35.0	22.0	21.0	21.5
2	27.0	29.0	28.0	23.0	23.0	23.0
3	29.0	27.0	28.0	25.0	22.0	23.5
4	33.0	27.0	30.0	22.0	27.0	24.5
5	40.0	31.0	35.5	26.0	22.0	24.0
6	28.0	33.0	30.5	23.0	25.0	24.0
7	29.0	28.0	28.5	28.0	26.0	27.0
8	35.0	28.0	31.5	22.0	23.0	22.5
9	27.0	27.0	27.0	22.0	23.0	22.5
10	30.0	29.0	29.5	23.0	24.0	23.5

Table 2: Summary Statistics under two connections

Connection	Mean	Standard Error	Standard Deviation	Coefficients Variation (CV)	Minimum	Maximum
3G	30.35	0.47	2.91	9.58	27.00	35.50
4G	23.60	0.92	1.49	6.30	21.50	27.00

Table 3. Raspberry pi camera features

Features	Camera module
Size	Around 25 × 24 × 9 mm
Weight	3g
Still resolution	5 Megapixels
Video modes	1080p30, 720p60 and 640 × 480p60/90
Linux integration	V4L2 driver available
Sensor	OmniVision OV5647
Sensor resolution	2592 × 1944 pixels
Pixel size	1.4 µm × 1.4 µm
Dynamic range	67 dB @ 8x gain
Sensitivity	680 mV/lux-sec

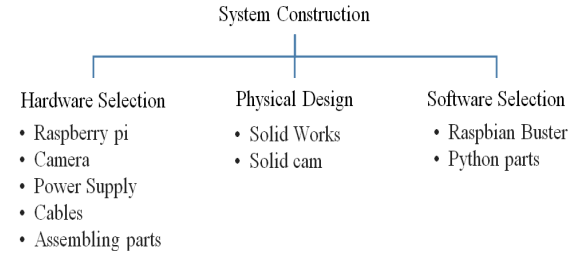

Figure 1: System Construction under different technologies

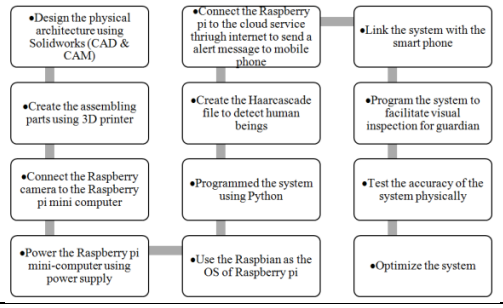

Figure 2: Flow diagram for system modeling

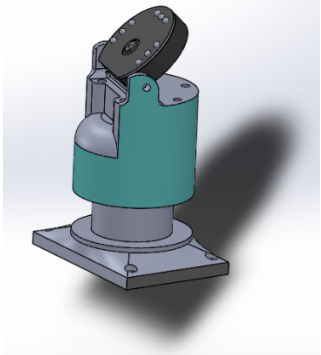

Figure 3. Camera Mount

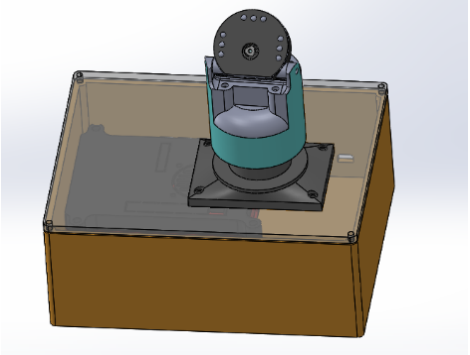

Figure 4. Camera Assembly

Figure 5. Wheelchair with Assembled System

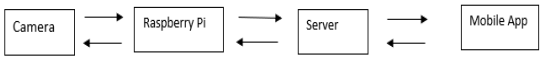

Figure 6: Block Diagram of Process

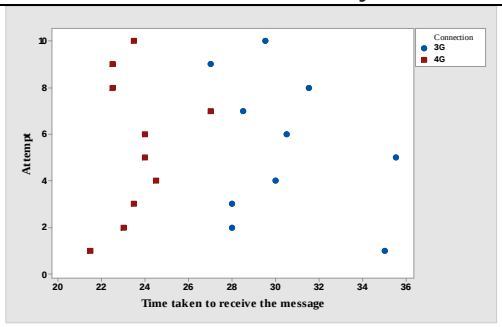

Figure 7: Scatter Plot of Attempt Vs. Time Taken to Receive the Message under 3G and 4G Connections

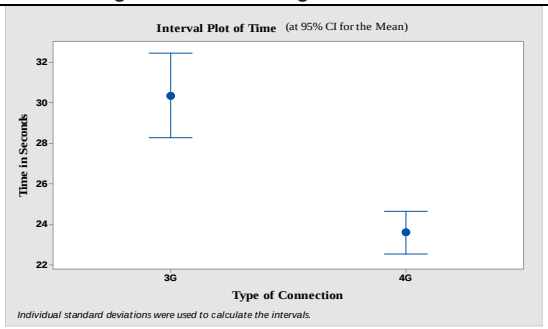

Figure 8: Interval plot of 3G and 4G connection at 95% level of Confidence

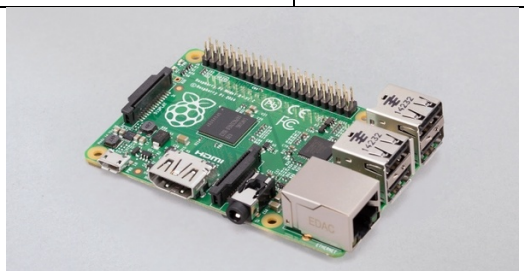

Figure 9: Raspberry Pi model B+

Appendix 1

Code: Classification algorithms

```
import cv2
import numpy
import os
import datetime
from twilio.rest import Client
face_cascade = cv2.CascadeClassifier('haarcascade_frontalface_default.xml')
cap=cv2.VideoCapture(0)
.
.
```

```
.

.
while True:
    ret,img=cap.read()
    gray=cv2.cvtColor(img,cv2.COLOR_BGR2GRAY)
    faces= face_cascade.detectMultiScale(gray)

    if (len(faces)) < 1 :
        number_T = number_T + 1
        print(number_T)
        if number_T == 100:
            number_K = 0
            currentDT = datetime.datetime.now()
            client.messages.create(body=' Alert message'+' '+str(currentDT),
                    from_=from_whatsapp_number,
                    to=to_whatsapp_number)
            number_T = 0
.
.
..
.
.
for(x,y,w,h) in faces:
        number_T = 0
        cv2.rectangle(img, (x, y), (x+w, y+h), (255, 0, 0), 2)
        roi_gray=gray[y:y+h,x:x+w]
        roi_color=img[y:y+h,x:x+w]

        cv2.imshow('img',img)
```

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RESEARCH ARTICLE

A Design of Modified Chain Sampling Plan MChSP-1 using Fuzzy Parameter

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ABSTRACT

This study deals with the concept of MChSP-1 using fuzzy parameter. FOC values for MChSP-1 are calculated. The design parameter of the sampling plan is determined by satisfying two risks at the specified quality levels. Then sum of the risks is also minimized for given & and optimum value of the sample size is obtained.

Key words: Modified Chain sampling plan, FOC curve, Fuzzy number, Trapezoidal fuzzy number

INTRODUCTION

Nowadays there is a heavy competition in business so it is important to maintain the quality of goods while manufacturing and marketing them. [12-13] SQC is a way of estimating the quality of a large number of items based on samples chosen from the larger group. The aim in production is to maintain the quality of the output. Zadeh [15] proposed a fuzzy set theory. It was especially developed to mathematically express uncertainty and ambiguity and to give formalized methods for dealing with the imprecision inherent in many issues. It is used to represent and manipulate data that is not exact. [16] Applications of fuzzy in many sectors of daily life, including engineering, medicine, meteorology, manufacturing, and others.

The OC curve and characteristics of chain sampling inspection plans are provided by Dodge [3]. For a chain sampling strategy, Clark [2] OC curves are used. The prolonged chain sampling plan of Frishman and Fred [7]. Dodge and Stephens [4] described a general family of chain sampling approach. Dodge and Stephens [5] created new

chain sampling inspection plans. Soundarajan's procedure and tables for developing and selecting a chain sampling strategy [14]. Govindaraju and Lia [8] created modified chain sampling strategies that always use the most recent lot-quality history. A lower sample size is required for the Mchsp-1 plan than for the zero acceptance number plan. Govindaraju and Subramani [9] provided selection of ChSP-1and ChSP(0,1) for given AQL and LQL. Fuzzy acceptance sampling plans for single and double sampling plans were developed by Kahraman and Kaya [10]. The outcome demonstrates that fuzzy parameters give greater flexibility and usefulness. Jamkhaneh and Sadeghpour Gildeh [1] created a chain sampling scheme (ChSP-1) using fuzzy probability theory . Turanoglu , Kaya and Kahraman [11] was studied OC curve using fuzzy parameters in acceptance sampling.

Basic definitions of fuzzy sets, operating procedure for MChSP-1, FOC curve values are generated using fuzzy parameter are all covered in this work. Sample size for given $\widetilde{AQL}$ & $\widetilde{LQL}$ is determined so as to satisfy both producer's and consumer's risks. The sum of the risks is minimized and the optimum value of the sample size is obtained and the results are provided in Tables.

Definition

Fuzzy Number (Zadeh [15] and Dubis & Prade [6]) : " Fuzzy set that are characterized on the arrangement of real numbers having the structure $\check{E}$: R tends to [0,1] are known as fuzzy number. A fuzzy number $\check{E}$ will be a fuzzy set in the real line that fulfills the state of both normal and convexity".

Trapezoidal fuzzy number Zadeh [15] and Dubis and Prade [6]**: "**If trapezoidal fuzzy numbers (TrFNs) are $\check{E}$ = (e_1, e_2, e_3, e_4) and its membership function as "

$$\text{TrFN} = \begin{cases} 0 & , \quad otherwise \\ \frac{y-e_1}{e_2-e_1} & , \ e_1 \le y \le e_2 \\ 1 & , \quad e_2 \le y \le e_3 \\ \frac{e_4-y}{e_4-e_3} & , \ e_3 \le y \le e_4 \\ 0 & , \quad otherwise \end{cases} \qquad \text{.....(1)}$$

"The interval of confidence of trapezoidal fuzzy number defined by γ cuts can be written as follows"

$$\check{E}[\gamma] = [e_1 + (e_2 - e_1)\gamma,\ e_4 - (e_4 - e_3)\gamma] \qquad \text{.....(2)}$$

Operating procedure of MChSP-1

According to Govindaraju and Lia [8] the operating procedure for MChSP-1 with various values for i =1, 2 and 3 and n.

Step 1: Draw a random sample of size n from entire lot and Count the no of defective units (d).

Step 2: If d=0 then accept the lot. No defective units was found in the previous i samples as well, except in one sample, i.e. d=1 is also acceptable.

Step 3: Reject the lot when d>1.

As a result, a Mchsp-1 plan contains parameters n , which is the sample size for each submitted lot, and i which is the number of prior samples to decide the result to acceptance or rejection

Fuzzy Probability of Acceptance

As per Jamkhaneh and Sadeghpour Gildeh [1] fuzzy probability of acceptance is calculated using fuzzy binomial distribution. γ cut of trapezoidal fuzzy number is used to solve MChSP-1 such that $\check{E}[\gamma] = [e_1 + (e_2 - e_1)\gamma,\ e_4 - (e_4 - e_3)\gamma]$ and taking γ=0,1 then we get fuzzy interval of proportion defective and interval value of fuzzy probability of acceptance $\widetilde{\wp}_s = (s, e_2 + s, e_3 + s, e_4 + s)$ Where $e_j = b_i - b_1$, i = 2,3,4 and $s \in [0, 1 - e_4]$ γ cuts of trapezoidal fuzzy number to find fuzzy operating characteristic curve where $\gamma = 0\ and\ \gamma = 1$. $\widetilde{\wp}_s[\gamma] = [s + e_2\gamma,\ e_4 + s - (e_4 - e_3)\gamma]$

As per Govindaraju and Lia [8], the fuzzy probability of acceptance for MChSP-1 is given by value of i =1,2,3.

$\mathcal{L}(\breve{\mathbb{P}}) = \mathbb{P}_0[\ (\mathbb{P}_0)^{i} + i(\mathbb{P}_0)^{i-1}\mathbb{P}_1]$ …. (3)

$\mathcal{L}(\breve{\wp}_s) = [(1-\breve{\wp}_s)^{n(i+1)} + i\, n\breve{\wp}_s(1-\breve{\wp}_s)^{n(i+1)-1}]$ ……. (4)

$\mathcal{L}(\breve{\wp}_s^{lb})[\gamma] = min[(1-\breve{\wp}_s)^{n(i+1)} + i\, n\breve{\wp}_s(1-\breve{\wp}_s)^{n(i+1)-1}]$ ……(5)

$\mathcal{L}(\breve{\wp}_s^{ub})[\gamma] = max[(1-\breve{\wp}_s)^{n(i+1)} + i\, n\breve{\wp}_s(1-\breve{\wp}_s)^{n(i+1)-1}]$ ……. (6)

$\mathcal{L}(\breve{\wp}_s)[\gamma] = [\mathcal{L}(\breve{\wp}_s^{lb}), \mathcal{L}(\breve{\wp}_s^{ub})]$ …... (7)

$= [(1-\breve{\wp}_s^{ub})^{n(i+1)} + i\, n\breve{\wp}_s^{ub}\ (1-\breve{\wp}_s^{ub})^{n(i+1)-1}, (1-\breve{\wp}_s^{lb})^{n(i+1)} + i\, n\breve{\wp}_s^{lb}\ (1-\breve{\wp}_s^{lb})^{n(i+1)-1}]$

The fuzzy probability of acceptance calculated for various values of the fuzzy proportion of defective and i =1,2,3 is provided in Table 1.one can observe that when the parameter 's' value is very small or nearer to zero then the fuzzy probability of acceptance approximately equal to unity.

Fuzzy Operating Characteristic curves

In the Figures 1& 2 , fuzzy proportion defective is plotted against the fuzzy probability of acceptance for γ=0 and γ=1. OC Curve has band with upper and lower bounds so that it is called as FOC Curve or band. When γ value increases from 0 to 1, FOC band value becomes closer.

Example 1

Suppose $\breve{\wp}_s$=(0.005,0.006.0.007,0.008) , n=20 and i = 1,2,3,4,5,6. one can obtain from Table 2 , fuzzy proportion defective for γ=0, $\breve{\wp}_s$ = [0.005 0.013] , and for γ=1, $\breve{\wp}_s$ = [0.011 0.012] then the fuzzy acceptance probability is calculated.

Fuzzy probability of acceptance when sample size varies

Let us assume that $\breve{\wp}_s$=(0.002,0.003,0.004,0.005) and the sample size n varies from 5 to 50 then γ cut of trapezoidal fuzzy number is used to calculate the interval of fuzzy proportion defective $\breve{\wp}_s[\gamma = 0]$ = [0.002 0.007] , $\breve{\wp}_s[\gamma = 1]$ = [0.005 0.006] and fuzzy probability of acceptance values as shown in the Table 3.when the sample size values decreases the width of FOC curve decreases.

Determination of sample size

As per Govindaraju and Subramani [9], MChSP-1 is used to design the sample size n to satisfy the following two inequalities for $\breve{\wp}_{2h}$ and $\mathcal{L}(\breve{\wp}_{2h})$ Simultaneously where $\breve{\wp}_{1f}$ is Acceptable quality level (AQL) and $\breve{\wp}_{2h}$ is Limiting quality level (LQL). Accepting the bad lot is called Consumer's risk ($\widetilde{\beta_h}$) and the rejecting the good lot is called producer's risk ($\widetilde{\alpha_f}$).

$\mathcal{L}(\breve{\wp}_{1f}) \geq 1-\widetilde{\alpha_f}$ and $\mathcal{L}(\breve{\wp}_{2h}) \leq \widetilde{\beta_h}$, Where $\widetilde{\alpha_f}$ = 0.05 and $\widetilde{\beta_h}$ = 0.10 is fixed so that the interval of fuzzy probability of acceptance is satisfied the conditions $\mathcal{L}(\breve{\wp}_{1f}) \geq 0.95$ and $\mathcal{L}(\breve{\wp}_{2h}) \leq 0.10$ for different sample sizes.

$\mathcal{L}(\breve{\wp}_{1f}) = [(1-\breve{\wp}_{1f})^{n(i+1)} + i n\breve{\wp}_{1f}(1-\breve{\wp}_{1f})^{n(i+1)-1}] \geq 0.95$ ……(8)

$\mathcal{L}(\breve{\wp}_{2h}) = [(1-\breve{\wp}_{2h})^{n(i+1)} + i n\breve{\wp}_{2h}(1-\breve{\wp}_{2h})^{n(i+1)-1}] \leq 0.10$ ……(9)

Minimizing the producer's risk and consumer's risk

The sample size is calculated so as to minimize the sum of the risks and it is presented in Table 5. where $\breve{\wp}_{1f}$ is AQL and $\breve{\wp}_{2h}$ is LQL and the corresponding the probability of acceptance values are $\mathcal{L}(\breve{\wp}_{1f})$ and $\mathcal{L}(\breve{\wp}_{2h})$. The mathematical expression to minimize the sum of risk is $\widetilde{\alpha_f} + \widetilde{\beta_h} = 1 - \mathcal{L}(\breve{\wp}_{1f}) + \mathcal{L}(\breve{\wp}_{2h}) = 1 - \left\{[(1-\breve{\wp}_{1f})^{n(i+1)} + in\breve{\wp}_{1f}(1-\breve{\wp}_{1f})^{n(i+1)-1}\right] + [(1-\breve{\wp}_{2h})^{n(i+1)} + in\breve{\wp}_{2h}(1-\breve{\wp}_{2h})^{n(i+1)-1}]\}$. The sum of risks is obtained as interval of fuzzy.

CONCLUSION

In this study a procedure of designing MChSP-1 using trapezoidal fuzzy number is presented. Fuzzy binomial distribution is used to calculate fuzzy probability of acceptance. FOC band values are calculated for the fixed sample size. One can note that fuzzy probability of acceptance is more for γ=0 than γ=1and FOC band is closer when γ=1. For γ=0, the sample size value is calculate such that it satisfied both the condition of producer's risk and consumer's risk. And also minimizing the sum of risks the optimum value sample size is obtained.

REFERENCES

1. E. Baloui Jamkhaneh and B. Sadeghpour Gildeh , "Chain sampling plan using fuzzy probability theory," Journal of applied sciences Vol.11, No.24, pp 3830-3838, 2011.
2. C.R. Clark, "OC Curves for ChSP-1 Chain Sampling Plans," Industrial Quality Control, Vol.17, No.4, pp.10-12, 1960.
3. H.F. Dodge, "Chain Sampling Inspection Plan," Industrial Quality Control, Vol.11, No.4 pp.10-13, 1955.
4. H.F. Dodge and K.S. Stephens, "A General Family of Chain Sampling Plans," Technical Report No. 20, Statistics center, Rutgers - The State University, Dec. 30, 1964.
5. H.F. Dodge and K.S. Stephens, "Some New Chain Sampling Inspection Plans," Industrial Quality Control, Vol.23, No.2, pp 61-67, 1966.
6. D.Dubis and H. Prade, "Operations of fuzzy Numbers", International Journal System Vol 9, pp 613-626, 1978.
7. Frishman and Fred, "An Extended chain sampling plan," Industrial Quality Control Vol.17, No.l, pp 10-12, 1960.
8. K. Govindaraju and Lia " A Modified Chsp-1 chain sampling plan , MChsp-1 with very small samples sizes", American Journal of Mathematics and Management science, 18 , 346-358, 1998.
9. K. Govindaraju and K Subramani, "Selection of Chain Sampling Plans ChSP-1 and ChSP-(0,l) for given Acceptable Quality Level and Limiting Quality Level," American Journal of Mathematical and Management Sciences, Vol. 13, No's 1 and 2, pp. 123-136 , 1993.
10. C. Kahraman and I. Kaya , "Fuzzy acceptance sampling plans, In C. Kahraman & M Yavuz, (Eds.), Production engineering and management under fuzziness pp. 457-481 springer 2010.
11. C. Kahraman and I. Kaya "Fuzzy acceptance sampling and characteristic curves," International journal of computational intelligence systems, Vol 5 No 1, pp 13-29, 2012.
12. Montgomery D.C, "Introduction to statistical quality control", Wiley, New York, USA, 1991.
13. Schiling E.G. " Acceptance sampling quality control", Dekker, New York, USA, 1982.
14. V. Soundararajan, "Procedures and Tables for Construction and Selection of Chain Sampling Plans (ChSP-1) - Part-I," Journal of Quality Technology, Vol.10, No.2, pp.56-60, 1978.
15. L. A. Zadeh, "Fuzzy sets", Information and control, 8, pp 338-353, 1965.
16. H.J. Zimmermann, "Fuzzy set theory and its applications", Kluwer academic Publishers, 1991.

Table 1 Fuzzy probability of acceptance with n=20

i	$\widetilde{\wp}_s = (s, e_2 + s, e_3 + s, e_4 + s)$	$\widetilde{\wp}_s[\gamma = 0]$	$\mathcal{L}(\widetilde{\wp}_{as})(\gamma = 0)$	$\widetilde{\wp}_s[\gamma = 1]$	$\mathcal{L}(\widetilde{\wp}_{as})(\gamma = 1)$
1	(0.000, 0.001, 0.002, 0.003)	[0 .000 0.003]	[1.0000 0.9401]	[0.001 0.002]	[0.9800 0.9600]
	(0.001, 0.002, 0.003, 0.004)	[0.001 0.005]	[0.9800 0.9006]	[0.003 0.004]	[0.9401 0.9203]
	(0.002, 0.003, 0.004, 0.005)	[0.002 0.007]	[0.9600 0.8615]	[0.005 0.006]	[0.9006 0.8810]
	(0.003, 0.004, 0.005, 0.006)	[0.003 0.009]	[0.9401 0.8231]	[0.007 0.008]	[0.8615 0.8422]
	(0.004, 0.005, 0.006, 0.007)	[0.004 0.011]	[0.9203 0.7854]	[0.009 0.010]	[0.8231 0.8041]
	(0.005, 0.006, 0.007, 0.008)	[0.005 0.013]	[0.9006 0.7486]	[0.011 0.012]	[0.7854 0.7669]
	(0.006, 0.007, 0.008, 0.009)	[0.006 0.015]	[0.8810 0.7127]	[0.013 0.014]	[0.7486 0.7305]
	(0.007, 0.008, 0.009, 0.01)	[0.007 0.017]	[0.8615 0.6779]	[0.015 0.016]	[0.7127 0.6952]
	(0.008, 0.009, 0.01, 0.011)	[0.008 0.019]	[0.8422 0.6441]	[0.017 0.018]	[0.6779 0.6608]
	(0.009, 0.010, 0.011, 0.012)	[0.009 0.021]	[0.8231 0.6114]	[0.019 0.020]	[0.6441 0.6276]
	(0.010, 0.011, 0.012, 0.013)	[0.010 0.023]	[0.8041 0.5799]	[0.021 0.022]	[0.6114 0.5955]
	(0.011, 0.012, 0.013, 0.014)	[0.011 0.025]	[0.7854 0.5495]	[0.023 0.024]	[0.5799 0.5646]
	(0.012, 0.013, 0.014, 0.015)	[0.012 0.027]	[0.7669 0.5203]	[0.025 0.027]	[0.5495 0.5347]
	(0.013, 0.014, 0.015, 0.016)	[0.013 0.029]	[0.7486 0.4922]	[0.027 0.028]	[0.5203 0.5061]
	(0.014, 0.015, 0.016, 0.017)	[0.014 0.031]	[0.7305 0.4653]	[0.029 0.030]	[0.4922 0.4786]
	(0.015, 0.016, 0.017, 0.018)	[0.015 0.033]	[0.7127 0.4396]	[0.031 0.032]	[0.4653 0.4523]
	(0.016, 0.017, 0.018, 0.019)	[0.016 0.035]	[0.6952 0.4149]	[0.033 0.034]	[0.4396 0.4271]
	(0.017, 0.018, 0.019, 0.02)	[0.017 0.037]	[0.6779 0.3914]	[0.035 0.036]	[0.4149 0.4030]
	(0.018, 0.019, 0.02, 0.021)	[0.018 0.039]	[0.6608 0.3690]	[0.037 0.038]	[0.3914 0.3801]
	(0.019, 0.02, 0.021, 0.022)	[0.019 0.041]	[0.6441 0.3476]	[0.039 0.040]	[0.3690 0.3582]
2	(0.000, 0.001, 0.002, 0.003)	[0 .000 0.003]	[1.0000 0.9356]	[0.001 0.002]	[0.9774 0.9579]
	(0.001, 0.002, 0.003, 0.004)	[0.001 0.005]	[0.9794 0.8910]	[0.003 0.004]	[0.9356 0.9126]
	(0.002, 0.003, 0.004, 0.005)	[0.002 0.007]	[0.9579 0.8411]	[0.005 0.006]	[0.8891 0.8652]
	(0.003, 0.004, 0.005, 0.006)	[0.003 0.009]	[0.9356 0.7925]	[0.007 0.008]	[0.8411 0.8168]
	(0.004, 0.005, 0.006, 0.007)	[0.004 0.011]	[0.9126 0.7441]	[0.009 0.010]	[0.7925 0.7682]
	(0.005, 0.006, 0.007, 0.008)	[0.005 0.013]	[0.8991 0.6963]	[0.011 0.012]	[0.7441 0.7201]
	(0.006, 0.007, 0.008, 0.009)	[0.006 0.015]	[0.8652 0.6498]	[0.013 0.014]	[0.6963 0.6729]
	(0.007, 0.008, 0.009, 0.01)	[0.007 0.017]	[0.8411 0.6047]	[0.015 0.016]	[0.6498 0.6270]
	(0.008, 0.009, 0.01, 0.011)	[0.008 0.019]	[0.8168 0.5614]	[0.017 0.018]	[0.6047 0.5828]
	(0.009, 0.010, 0.011, 0.012)	[0.009 0.021]	[0.7925 0.5200]	[0.019 0.020]	[0.5614 0.5405]
	(0.010, 0.011, 0.012, 0.013)	[0.010 0.023]	[0.7682 0.4807]	[0.021 0.022]	[0.5200 0.5001]
	(0.011, 0.012, 0.013, 0.014)	[0.011 0.025]	[0.7441 0.4434]	[0.023 0.024]	[0.4807 0.4618]
	(0.012, 0.013, 0.014, 0.015)	[0.012 0.027]	[0.7201 0.4084]	[0.025 0.027]	[0.4434 0.4256]
	(0.013, 0.014, 0.015, 0.016)	[0.013 0.029]	[0.6963 0.3754]	[0.027 0.028]	[0.4084 0.3916]
	(0.014, 0.015, 0.016, 0.017)	[0.014 0.031]	[0.6729 0.3446]	[0.029 0.030]	[0.3754 0.3597]
	(0.015, 0.016, 0.017, 0.018)	[0.015 0.033]	[0.6498 0.3158]	[0.031 0.032]	[0.3446 0.3299]
	(0.016, 0.017, 0.018, 0.019)	[0.016 0.035]	[0.6270 0.2890]	[0.033 0.034]	[0.3158 0.3022]
	(0.017, 0.018, 0.019, 0.02)	[0.017 0.037]	[0.6047 0.2642]	[0.035 0.036]	[0.2890 0.2764]
	(0.018, 0.019, 0.02, 0.021)	[0.018 0.039]	[0.5828 0.2411]	[0.037 0.038]	[0.2642 0.2524]
	(0.019, 0.02, 0.021, 0.022)	[0.019 0.041]	[0.5614 0.2198]	[0.039 0.040]	[0.2411 0.2303]
3	(0.000, 0.001, 0.002, 0.003)	[0 .000 0.003]	[1.0000 0.9283]	[0.001 0.002]	[0.9785 0.9545]
	(0.001, 0.002, 0.003, 0.004)	[0.001 0.005]	[0.9785 0.8716]	[0.003 0.004]	[0.9283 0.9005]
	(0.002, 0.003, 0.004, 0.005)	[0.002 0.007]	[0.9545 0.8112]	[0.005 0.006]	[0.8716 0.8417]
	(0.003, 0.004, 0.005, 0.006)	[0.003 0.009]	[0.9283 0.7495]	[0.007 0.008]	[0.8112 0.7804]
	(0.004, 0.005, 0.006, 0.007)	[0.004 0.011]	[0.9005 0.6882]	[0.009 0.010]	[0.7495 0.7187]
	(0.005, 0.006, 0.007, 0.008)	[0.005 0.013]	[0.8716 0.6285]	[0.011 0.012]	[0.6882 0.6581]
	(0.006, 0.007, 0.008, 0.009)	[0.006 0.015]	[0.8417 0.5712]	[0.013 0.014]	[0.6285 0.5995]
	(0.007, 0.008, 0.009, 0.01)	[0.007 0.017]	[0.8112 0.5169]	[0.015 0.016]	[0.5712 0.5436]
	(0.008, 0.009, 0.01, 0.011)	[0.008 0.019]	[0.7804 0.4660]	[0.017 0.018]	[0.5169 0.4910]
	(0.009, 0.010, 0.011, 0.012)	[0.009 0.021]	[0.7495 0.4187]	[0.019 0.020]	[0.4660 0.4419]

	(0.010, 0.011, 0.012, 0.013)	[0.010 0.023]	[0.7187 0.3750]	[0.021 0.022]	[0.4187 0.3964]
	(0.011, 0.012, 0.013, 0.014)	[0.011 0.025]	[0.6882 0.3349]	[0.023 0.024]	[0.3750 0.3545]
	(0.012, 0.013, 0.014, 0.015)	[0.012 0.027]	[0.6581 0.2983]	[0.025 0.027]	[0.3349 0.3162]
	(0.013, 0.014, 0.015, 0.016)	[0.013 0.029]	[0.6285 0.2651]	[0.027 0.028]	[0.2983 0.2813]
	(0.014, 0.015, 0.016, 0.017)	[0.014 0.031]	[0.5995 0.2351]	[0.029 0.030]	[0.2651 0.2497]
	(0.015, 0.016, 0.017, 0.018)	[0.015 0.033]	[0.5712 0.2080]	[0.031 0.032]	[0.2351 0.2212]
	(0.016, 0.017, 0.018, 0.019)	[0.016 0.035]	[0.5436 0.1837]	[0.033 0.034]	[0.2080 0.1955]
	(0.017, 0.018, 0.019, 0.02)	[0.017 0.037]	[0.5169 0.1619]	[0.035 0.036]	[0.1837 0.1725]
	(0.018, 0.019, 0.02, 0.021)	[0.018 0.039]	[0.4910 0.1425]	[0.037 0.038]	[0.1619 0.1519]
	(0.019, 0.02, 0.021, 0.022)	[0.019 0.041]	[0.4660 0.1252]	[0.039 0.040]	[0.1425 0.1336]

Table 2. Fuzzy probability of acceptance where i =1 to 6 and n=20

i	$\mathcal{L}(\tilde{\wp}_{as})(\gamma = 0)$	$\mathcal{L}(\tilde{\wp}_{as})(\gamma = 1)$
1	[0.9600 0.8615]	[0.9006 0.8810]
2	[0.9579 0.8411]	[0.8891 0.8652]
3	[0.9545 0.8112]	[0.8761 0.8417]
4	[0.9498 0.7747]	[0.8493 0.8124]
5	[0.9440 0.7339]	[0.8234 0.7789]
6	[0.9373 0.6904]	[0.7946 0.7425]

Table 3: Fuzzy probability of acceptance when sample size varies

n	$\mathcal{L}(\tilde{\wp}_{as})(\gamma = 0)$	$\mathcal{L}(\tilde{\wp}_{as})(\gamma = 1)$
50	[0.8715 0.5049]	[0.6436 0.5718]
45	[0.8861 0.5511]	[0.6808 0.6143]
40	[0.9005 0.5999]	[0.7188 0.6583]
35	[0.9146 0.6509]	[0.7573 0.7035]
30	[0.9283 0.7035]	[0.7958 0.7496]
25	[0.9416 0.7573]	[0.8341 0.7958]
20	[0.9545 0.8112]	[0.8716 0.8417]
15	[0.9668 0.8642]	[0.9077 0.8862]
10	[0.9785 0.9147]	[0.9417 0.9284]
5	[0.9896 0.9608]	[0.9728 0.9669]

Table 4: Optimum parameter n, when $\mathcal{L}(\tilde{\wp}_{1f}) \geq 0.95$ and $\mathcal{L}(\tilde{\wp}_{2h}) \leq 0.10$

i	$(\widetilde{AQL})$	$(\widetilde{LQL})$	n
1	(0.001,0.0011,0.0012,0.0013)	(0.05,0.051,0.052,0.053)	35
	(0.001,0.0011,0.0012,0.0013)	(0.06,0.061,0.062,0.063)	35
	(0.001,0.0011,0.0012,0.0013)	(0.07.0.071,0.072,0.073)	35
	(0.001,0.0011,0.0012,0.0013)	(0.08,0.081,0.082,0.083)	30
	(0.001,0.0011,0.0012,0.0013)	(0.09,0.091,0.092,0.093)	30
	(0.002,0.0021,0.0022,0.0023)	(0.08,0.081,0.082,0.083)	20
	(0.002,0.0021,0.0022,0.0023)	(0.09,0.091,0.092,0.093)	18
	(0.003,0.0031,0.0032,0.0033)	(0.09,0.091,0.092,0.093)	14
2	(0.001,0.0011,0.0012,0.0013)	(0.05,0.051,0.052,0.053)	36
	(0.001,0.0011,0.0012,0.0013)	(0.06,0.061,0.062,0.063)	34
	(0.001,0.0011,0.0012,0.0013)	(0.07.0.071,0.072,0.073)	34
	(0.001,0.0011,0.0012,0.0013)	(0.08,0.081,0.082,0.083)	30
	(0.001,0.0011,0.0012,0.0013)	(0.09,0.091,0.092,0.093)	30

	(0.002,0.0021,0.0022,0.0023)	(0.05,0.051,0.052,0.053)	20
	(0.002,0.0021,0.0022,0.0023)	(0.06,0.061,0.062,0.063)	20
	(0.002,0.0021,0.0022,0.0023)	(0.07.0.071,0.072,0.073)	16
	(0.002,0.0021,0.0022,0.0023)	(0.08,0.081,0.082,0.083)	18
	(0.002,0.0021,0.0022,0.0023)	(0.09,0.091,0.092,0.093)	20
	(0.003,0.0031,0.0032,0.0033)	(0.07.0.071,0.072,0.073)	13
	(0.003,0.0031,0.0032,0.0033)	(0.08,0.081,0.082,0.083)	12
	(0.003,0.0031,0.0032,0.0033)	(0.09,0.091,0.092,0.093)	12
	(0.004,0.0041,0.0042,0.0043)	(0.09,0.091,0.092,0.093)	10
3	(0.001,0.0011,0.0012,0.0013)	(0.05,0.051,0.052,0.053)	30
	(0.001,0.0011,0.0012,0.0013)	(0.06,0.061,0.062,0.063)	25
	(0.001,0.0011,0.0012,0.0013)	(0.07.0.071,0.072,0.073)	30
	(0.001,0.0011,0.0012,0.0013)	(0.08,0.081,0.082,0.083)	25
	(0.001,0.0011,0.0012,0.0013)	(0.09,0.091,0.092,0.093)	20
	(0.002,0.0021,0.0022,0.0023)	(0.05,0.051,0.052,0.053)	17
	(0.002,0.0021,0.0022,0.0023)	(0.06,0.061,0.062,0.063)	15
	(0.002,0.0021,0.0022,0.0023)	(0.07.0.071,0.072,0.073)	17
	(0.002,0.0021,0.0022,0.0023)	(0.08,0.081,0.082,0.083)	15
	(0.002,0.0021,0.0022,0.0023)	(0.09,0.091,0.092,0.093)	15
	(0.003,0.0031,0.0032,0.0033)	(0.06,0.061,0.062,0.063)	13
	(0.003,0.0031,0.0032,0.0033)	(0.07.0.071,0.072,0.073)	13
	(0.003,0.0031,0.0032,0.0033)	(0.08,0.081,0.082,0.083)	13
	(0.003,0.0031,0.0032,0.0033)	(0.09,0.091,0.092,0.093)	13

Table 5: Optimum parameter n and the minimum sum of the risks $when\ \widetilde{\alpha_f} \cong 0.05\ and\ \widetilde{\beta_h} \cong 0.10$

i	n	$\breve{\wp}_{1f}\ [\gamma = 0]$	$\mathcal{L}(\breve{\wp}_{1f})[\gamma = 0]$	$\breve{\wp}_{2h}\ [\gamma = 0]$	$\mathcal{L}(\breve{\wp}_{2h})[\gamma = 0]$	$\widetilde{\alpha_f} + \widetilde{\beta_h}$
1	30	[0.001 0.0013]	[0.9700 0.9546]	[0.05 0.053]	[0.1188 0.1021]	[0.1488 0.1475]
	30	[0.001 0.0013]	[[0.9700 0.9546]	[0.06 0.063]	[0.0712 0.0608]	[0.1012 0.1062]
	35	[0.001 0.0013]	[0.9650 0.9546]	[0.07 0.073]	[0.0440 0.0186]	[0.079 0.064]
	35	[0.001 0.0013]	[0.9650 0.9546]	[0.08 0.083]	[0.0118 0.0097]	[0.0468 0.0551]
	35	[0.001 0.0013]	[0.9650 0.9546]	[0.09 0.093]	[0.0061 0.0049]	[0.0411 0.0503]
	20	[0.002 0.0023]	[0.9600 0.9541]	[0.08 0.083]	[0.0975 0.0878]	[0.1375 0.1337]
	18	[0.002 0.0023]	[0.9640 0.9632]	[0.09 0.093]	[0.0932 0 .0847]	[0.1292 0.1215]
	14	[0.003 0.0033]	[0.9580 0.9539]	[0.09 0.093]	[0.1701 0.583]	[0.2121 0.2044]
	36	[0.001 0.0013]	[0.9623 0.9504]	[0.05 0.053]	[0.0188 0.0140]	[0.0565 0.0636]
	34	[0.001 0.0013]	[0.9644 0.9533]	[0.06 0.063]	[0.0097 0.0073]	[0.0453 0.0540]
	34	[0.001 0.0013]	[0.9644 0.9533]	[0.07 0.073]	[0.0037 0.0028]	[0.0393 0.0495]
	30	[0.001 0.0013]	[0.9688 0.9590]	[0.08 0.083]	[0.0034 0.0026]	[0.0613 0.0655]
	30	[0.001 0.0013]	[0.9688 0.9590]	[0.09 0.093]	[0.0014 0.0011]	[0.0326 0.0421]
	20	[0.002 0.0023]	[0.9579 0.9513]	[0.05 0.053]	[0.1431 0.1234]	[0.1852 0.1721]
	18	[0.002 0.0023]	[0.9623 0.9564]	[0.06 0.063]	[0.1167 0.1019]	[0.1544 0.1455]
	16	[0.002 0.0023]	[0.9666 0.9614]	[0.07 0.073]	[0.1047 0.0925]	[0.1381 0.1311]
	18	[0.002 0.0023]	[0.9623 0.9564]	[0.08 0.083]	[0.0458 0.0396]	[0.0835 0.0832]
	20	[0.002 0.0023]	[0.9579 0.9513]	[0.09 0.093]	[0.0173 0 .1632]	[0.0378 0.0633]
	13	[0.003 0.0033]	[0.9590 0.9547]	[0.07 0.073]	[0.1745 0.1585]	[0.2155 0.2038]
	12	[0.003 0.0033]	[0.9623 0.9584]	[0.08 0.083]	[0.1534 0.1402]	[0.1911 0.1818]

	12	[0.003 0.0033]	[0.9623 0.9584]	[0.09 0.093]	[0.1131 0.1030]	[0.1508 0.1446]
3	30	[0.001 0.0013]	[0.9668 0.9557]	[0.05 0.053]	[0.0122 0.0088]	[0.0434 0.0531]
	25	[0.001 0.0013]	[0.9727 0.9637]	[0.06 0.063]	[0.0119 0.0090]	[0.0392 0.0453]
	30	[0.001 0.0013]	[0.9668 0.9557]	[0.07 0.073]	[0.0013 0.0009]	[0.0325 0.0452]
	25	[0.001 0.0013]	[0.9727 0.9637]	[0.08 0.083]	[0.0018 0.0013]	[0.0291 0.0376]
	20	[0.001 0.0013]	[0.9785 0.9715]	[0.09 0.093]	[0.0037 0.0029]	[0.0279 0.0368]
	17	[0.002 0.0023]	[0.9619 0.9556]	[0.05 0.053]	[0.1126 0.0950]	[0.1507 0.1394]
	15	[0.002 0.0023]	[0.9668 0.9613]	[0.06 0.063]	[0.0945 0.0811]	[0.1277 0.1198]
	17	[0.002 0.0023]	[0.9619 0.9556]	[0.07 0.073]	[0.0348 0.0290]	[0.0729 0.0734]
	15	[0.002 0.0023]	[0.9668 0.9613]	[0.08 0.083]	[0.0330 0.0280]	[0.0662 0.0667]
	15	[0.002 0.0023]	[0.9668 0.9613]	[0.09 0.093]	[0.0190 0.0161]	[0.0502 0.0548]
	13	[0.003 0.0033]	[0.9557 0.9508]	[0.06 0.063]	[0.1398 0.1229]	[0.1841 0.1721]
	13	[0.003 0.0033]	[0.9557 0.9508]	[0.07 0.073]	[0.1369 0.0790]	[0.1812 0.1282]
	13	[0.003 0.0033]	[0.9557 0.9508]	[0.08 0.083]	[0.0575 0.0500]	[0.1018 0.0992]
	13	[0.003 0.0033]	[0.9557 0.9508]	[0.09 0.093]	[0.0360 0.0312]	[0.0803 0.0804]

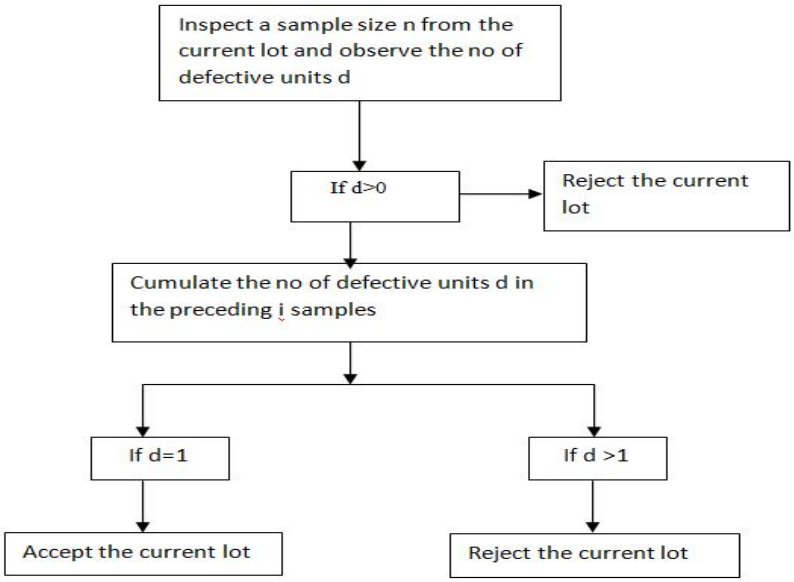

Fig.1.The flow chart for operating procedure of MChSP-1

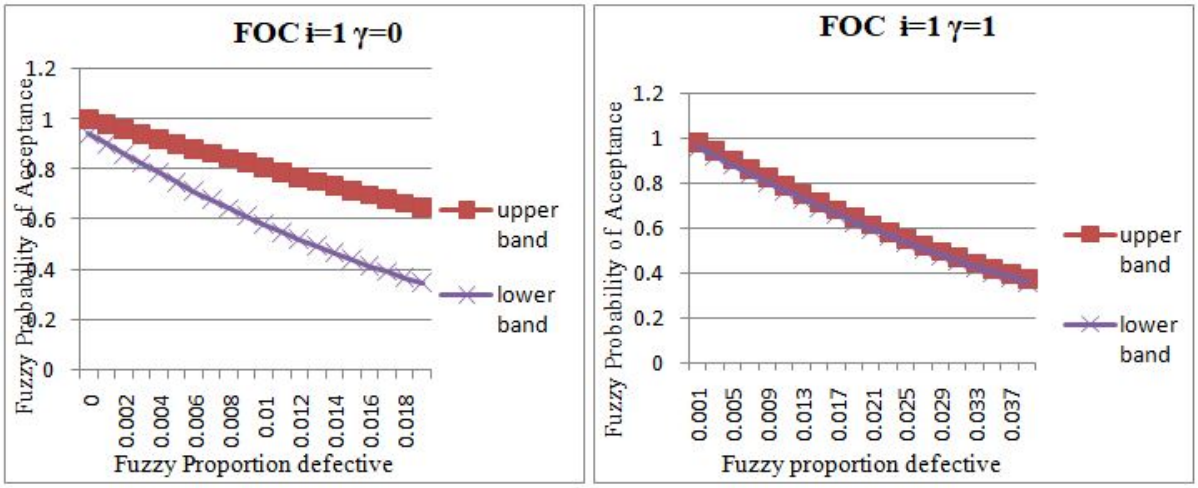

Fig. 2 & 3 Fuzzy Operating Characteristic curve for MChSP-1

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RESEARCH ARTICLE

Micronutrient Status in Patients with Metabolic Syndrome

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ABSTRACT

Metabolic syndrome (Mets) is a chronic metabolic condition that may impair an individual's nutritional status. Certain micronutrients, particularly zinc and magnesium, have been linked to the development of glucose intolerance. This research aims to determine the quality of different micronutrients, such as vitamins and minerals, in people with metabolic syndrome and healthy adults. In addition, the function of these micronutrients in insulin secretion and carbohydrate metabolism is addressed to establish if reported changes in mineral or vitamin concentration in blood or tissue contribute to Mets patients' carbohydrate intolerance. The authors conclude that metabolic syndrome may cause alterations in the levels of essential micronutrients. However, adequately controlled research should be conducted to determine the involvement of trace elements in the pathophysiology of Mets.

Keywords: Metabolic syndrome, Micronutrients, Cardiovascular disease, Biomarkers, Trace elements

INTRODUCTION

Numerous research conducted over the past two decades has discovered changes in patients' micronutrient status with metabolic syndrome [1]. In some instances, lack of specific minerals or vitamins has been linked to different problems. As early as the 16th century, the complex connection between nutrition and Mets was suspected [2]. However, uncertainty in the methodology and variations in the patient groups examined have resulted in conflicting results and contentious conclusions. Additionally, the stringent dietary restrictions advised for obese diabetics and the effect of newly recommended high-fibre diets on mineral and vitamin absorption cause to worry [3].Despite the

extensive research on the importance of food composition in Mets management, there are comparatively few investigations on the impact of Mets on an individual's nutritional status [4].However, the precise significance of malnutrition in Mets' pathogenesis has been debated [5].This study aims to determine the vitamin and mineral status of individuals with diabetes mellitus and determine if these substances may have a role in the etiology of Mets.

MATERIALS AND METHODS

A microbiological test kit was used to estimate vitamin B6 levels in serum. Folic acid is a kind of vitamin (Vitamin B9) The Roche Elecsys 2010 immunoassay analyzer was used to measure folic acid. ADVIA Centaur (Bayer Diagnostics/Seimen Healthcare Diagnostics) tested vitamin B12. 25 - Hydroxy Vitamin D: Vit-D evaluated by the Architect 25-OH Vit-D System (Abbott). The ARCHITECT 25-OH Vit-D test is a chemiluminescent micro particle immunoassay (CMIA) for measuring 25-hydroxyvitamin D (25-OH vitamin D) in human blood and plasma. Ascorbic acid was determined chemically using the DTC reagent (2, 4-dinitrophenyl hydrazine-thiourea-CuSO reagent), and VITROS Mg. Magnesium (Mg) concentrations in serum, plasma, and urine are quantified using slides. Competitive immunoassay employing chemiluminescence was used to assess serum tetra-iodothyronine.

RESULTS AND DISCUSSION

Diagram 1 and Table 1 depict statistics of the micronutrients in healthy adults and individuals with Mets. Significant changes are observed in Mets patients, which may be responsible for the metabolic alterations. Certain trace element concentrations in the serum were observed to be changed in Mets patients compared to controls. In Mets patients, serum ascorbic acid, group B vitamins, and 1, 25 dihydroxycholecalciferol concentrations were found to be low. Detailed descriptive statistic shown in the table 1.

Comparison of the serum levels of micronutrients in subjects with and without metabolic syndrome

Table 2 illustrates the Mann-Whitney test for comparing blood micronutrient levels in individuals with and without metabolic syndrome. Vitamin D has a Z statistic of -13.191 and a p-value of 0.000. We reject the null hypothesis since the p-value is less than 0.05 and conclude that people with and without metabolic syndrome have significantly different blood Vitamin D levels. Vitamin C has a Z statistic of -6.411 and a p-value of 0.000. Because the p-value is less than 0.05, we reject the null hypothesis and conclude that there is a significant difference in blood Vitamin C levels between individuals with and without metabolic syndrome. Folate has a Z statistic of -2.908 and a p-value of 0.004. Because the p-value is less than 0.05, we reject the null hypothesis and conclude that there is a statistically significant difference in Folate serum levels between individuals with and without metabolic syndrome. Vitamin B6 PLP has a Z statistic of -1.334 and a p-value of 0.182. Because the p-value is higher than 0.05, we accept the null hypothesis and conclude that there is no significant difference in blood Vitamin B6 PLP levels between individuals with and without metabolic syndrome. Vitamin B12 has a Z statistic of -2.149 and a p-value of 0.032. Because the p-value is less than 0.05, we reject the null hypothesis and conclude that there is a significant difference in blood Vitamin B12 levels between individuals with and without metabolic syndrome. Magnesium has a p-value of 0.558 and a Z statistic of -0.585. Because the p-value is higher than 0.05, we accept the null hypothesis and conclude that there is no statistically significant difference in Magnesium serum levels between individuals with and without metabolic syndrome. Zinc's Z statistic is -3.095, and its p-value is 0.002. Because the p-value is less than 0.05, we reject the null hypothesis and conclude that there is a statistically significant difference in zinc serum levels between individuals with and without metabolic syndrome. Calcium has a p-value of 0.054 and a Z statistic of -1.928. Because the p-value is higher than 0.05, we accept the null hypothesis and conclude that there is no significant difference in blood calcium levels between individuals with and without metabolic syndrome. The Z statistic for phosphorus is -2.458, and the p-value is 0.014. Because the p-value is less than 0.05, we reject the null hypothesis and conclude a significant difference in Phosphorus serum levels between individuals with and without metabolic syndrome.

Investigations have shown that deficits in a variety of trace minerals, including zinc and magnesium and vitamin C and B-6, may result in glucose intolerance. However, there are currently no correctly controlled studies demonstrating the involvement of trace elements in the development of carbohydrate intolerance. Although most Mets patients do not have micronutrient deficiencies, a subset of individuals has been found to have zinc and magnesium deficits.

CONCLUSION

The authors conclude that metabolic syndrome may cause changes in key micronutrient levels. However, adequately controlled research should be carried out to determine the role of trace elements in Mets pathophysiology.

REFERENCES

1. Shahinfar H, Akbarzade Z, Djafari F, Shab-Bidar S. Association of nutrient patterns and metabolic syndrome and its components in adults living in Tehran, Iran. Journal of Diabetes& Metabolic Disorders. 2020 ;19(2):1071-9.
2. Jepsen S, Suvan J, Deschner J. The association of periodontal diseases with metabolic syndrome and obesity. Periodontology 2000. 2020 Jun;83(1):125-53.
3. Awuchi CG, Igwe VS, Amagwula IO. Nutritional diseases and nutrient toxicities: A systematic review of the diets and nutrition for prevention and treatment. International Journal of Advanced Academic Research. 2020;6(1):1-46.
4. Serbis A, Giapros V, Galli-Tsinopoulou A, Siomou E. Metabolic Syndrome in Children and Adolescents: Is There a Universally Accepted Definition? Does it Matter?. Metabolic Syndrome and Related Disorders. 2020 Dec 1;18(10):462-70.
5. Misra A, Soares MJ, Mohan V, Anoop S, Abhishek V, Vaidya R, Pradeepa R. Body fat, metabolic syndrome and hyperglycemia in South Asians. Journal of diabetes and its complications. 2018 Nov 1;32(11):1068-75.

Table 1: Descriptive Statistics of the micronutrients

		N	Mean	Std. Deviation
Vitamin D (ng/mL)	Experimental	120	15.9025	4.58563
	Control	120	32.1767	2.24199
Vitamin C (mg/dl)	Experimental	120	.8267	.27676
	Control	120	1.1708	.41534
Folate (5–20 ng/ml)	Experimental	120	14.5750	2.79755
	Control	120	15.5500	2.67214
Vitamin B6 PLP (5 - 50 µg/L)	Experimental	120	27.2750	9.21723
	Control	120	28.9750	9.06509
Vitamin B12 (20–80 ng/dl)	Experimental	120	53.7167	15.04122
	Control	120	57.8000	12.59558
Magnesium (1.8–2.2 mg/dl)	Experimental	120	1.8250	.16049
	Control	120	1.8375	.14785
Zinc (50–100 ?g/dl)	Experimental	120	75.6583	12.42084
	Control	120	80.5750	10.54854
Calcium (mg/dl)	Experimental	120	9.3017	.56420
	Control	120	9.4217	.52950
Phosphorus (mg/dl)	Experimental	120	4.3667	.58859
	Control	120	4.5292	.47640

Table 2. Mann-Whitney test for comparison of serum levels of micronutrients in subjects with and without metabolic syndrome

Test Statistics	Mann-Whitney U	Wilcoxon W	Z	p-value
Vitamin D (ng/mL)	107.500	7367.500	-13.191	0.000
Vitamin C (mg/dl)	3771.500	11031.500	-6.411	0.000
Folate (5–20 ng/ml)	5649.000	12909.000	-2.908	0.004
Vitamin B6 PLP (5 - 50 µg/L)	6483.500	13743.500	-1.334	0.182
Vitamin B12 (20–80 ng/dl)	6046.000	13306.000	-2.149	0.032
Magnesium (1.8–2.2 mg/dl)	6901.500	14161.500	-0.585	0.558
Zinc (50–100 µg/dl)	5538.500	12798.500	-3.095	0.002
Calcium (mg/dl)	6167.500	13427.500	-1.928	0.054
Phosphorus (mg/dl)	5881.500	13141.500	-2.458	0.014

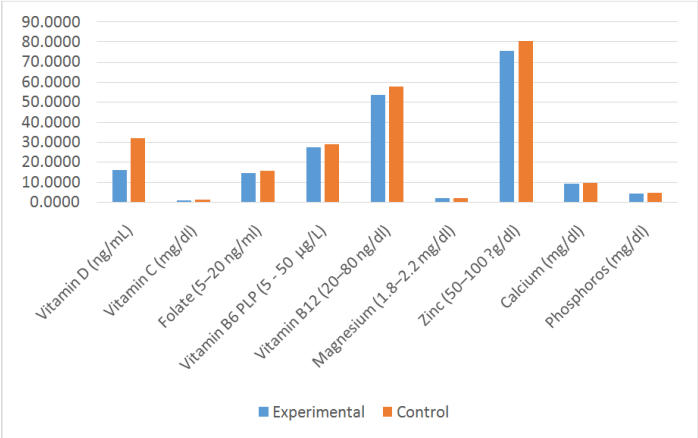

Figure 1. Diagram showing the average levels of micronutrients

Instruction to author

INSTRUCTION TO AUTHOR, 2021

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Articles in Journals

1. Devi KV, Pai RS. Antiretrovirals: Need for an Effective Drug Delivery. Indian J Pharm Sci 2006;68:1-6.
List the first six contributors followed by *et al*.

2. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.

3. Issue with supplement: Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1, Suppl 2):89-97.

Books and other Monographs

4. Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

5. Editor(s), compiler(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

6. Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

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RESEARCH ARTICLE

Anti-cyclic Citrullinated Peptide Antibody and Rheumatoid Factor in Rheumatoid Arthritis Patients: Evaluation of Clinical Relevance

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ABSTRACT

Rheumatoid Arthritis (RA) is a prevalent Systemic Autoimmune Disorder with varying degrees of morbidity and mortality. Immunoassays based on synthetic citrullinated peptide are highly successful for detecting auto antibodies against cyclic citrullinated peptide, which is a recent and highly specific marker for RA in addition to Rheumatoid factor (RF). C Reactive protein (CRP) levels are also linked to inflammatory processes in RA patients. This research attempts to investigate and compare the frequency and interaction of anti-cyclic citrullinated peptide antibody (ACCP), RF, and CRP in RA suspects. The aim of this research is to evaluate the role and critically assess the degree of sensitivity, precision, a positive and negative anticipating worth of ACCP & RF in rheumatoid arthritis patients. Specimens from RA people were assessed for RF, Anti-CCP and CRP by particular immunologic and also photometric assays. ACCP has a higher specificity for RA in the study, while RF has a higher degree of sensitivity. According to the findings of this review, while ACCP assays provide enhanced uniqueness for RA, RF has a higher degree of sensitivity. The combination of traditional and novel biomarkers may be useful in predicting and diagnosing medical conditions at an early stage, as a result, it is advised to use a combination of ACCP and RF for the diagnosis of RA, as it provides clinical sensitivity close to 100 percent.

Keywords: Rheumatoid Arthritis, Rheumatoid factor, Systemic Lupus Erythematosus, Anticyclic citrullinated peptide, Biomarkers.

INTRODUCTION

Usually, the rheumatoid factor (RF) and the anti-cyclic citrullinated peptide (anti-CCP) are used for RA diagnosis under the EULAR 2010 guidelines. Since 2010, new biomarkers have been identified and have proven useful in early-stage RA detection. One of the main phases in the evolution of patients with RA is to identify and differentiate the more acute manifestations of the disorder by prognostic biomarkers for more comprehensive treatment of these events. In order to direct the clinical and therapeutic management of all phases of rheumatoid arthritis, biomarkers are critical because they can help predict the progression of disease in subjects at risk, enhance diagnosis by closing the serological gap, provide prognostic knowledge that is useful for making therapeutic decisions and assessing responses and effects of care, and allow disease behaviour and progress Before the initiation of signs such as rheumatoid factor and anti-citrullinated protein antibodies.

Rheumatoid Arthritis Disease is a major autoimmune diseases. These ailments are linked to a variety of immunological, metabolic, hereditary, and haematological abnormalities. Just a few Indian-based studies have looked into the efficacy of these biomarkers and their ties to treatment of these issues. RA is an inflammatory disorder that necessitates the use of biomarkers in order to properly treat patients. Some of these markers aid in diagnosis, while others examine the role of the issue and the result of treatment. Because of the diversity of clinical discussion as well as the disparity in the distribution of biomarkers in different clients, combinations of normal and even novel biomarkers may be useful in very early diagnosis and therapy.

Since 2010, new biomarkers have been identified and demonstrated to be useful for early-stage RA medical diagnosis. Numerous biomarkers may be used to identify subjects at risk of rheumatoid arthritis and even those vulnerable to pre-clinical rheumatoid arthritis up until the onset of signs and symptoms such as rheumatoid component and anti-citrullinated safe protein antibodies. Biomarkers such as the cost of erythrocyte sedimentation and the amount of C-reactive safe protein provide information on the progression of the disease. Predictive biomarkers, on the other hand, enable medical professionals to identify the capacity for therapy response, particularly in the age of organic drugs, prior to beginning a specific treatment.

MATERIALS AND METHODS

5 mL of venous blood was taken from all the patients after obtaining their educated approval, product apart and were evaluated for ACCP by 2nd generation Chemiluminescence. RF as well as CRP degrees were assessed by Immuno-nephelometry.

RESULTS AND DISCUSSION

Blood samples from rheumatoid arthritis patients were analyzed for RF and Anti-CC serological markers. The practitioner presented the illness incidence rankings to the customers. The aim of this analysis was to determine the sensitivity, accuracy, and positive and negative predictive value of anti-cyclic citrullinated peptide in rheumatoid arthritis patients. Numerous investigators have reported parallel searches, as the most recent disease-related research survey focuses mainly on the midlife population, with a female majority. Reciprocal joint pain or irritability is widely accepted as one of the most persistent signs and symptoms in persons with rheumatoid joint inflammation, and the majority may feel multiple joints. At the rheumatic clinic, a greater number of patients experienced bilateral pain and mild joint swelling. Medical synovitis is a key symptom of rheumatoid arthritis which should be taken into account by doctors when treating patients with rheumatoid arthritis. Anti-CCP antibodies have a general susceptibility of 40%, a 100% positive anticipating value, and a 60% negative anticipating value. Anti-CCP antibody is associated with an increased risk of knee pain and joint pain in proportion to joint involvement, increased CRP, and rheumatoid

factor. Females were shown to have a higher prevalence of rheumatoid arthritis (80%) than males. RF, which was formerly used as a diagnostic and prognostic marker, is insufficiently specific. The present research discovered RF and CRP positivity in normal stable control groups, showing the need for a more specific test for the diagnosis and prognosis of rheumatoid arthritis. The study found that ACCP has a higher specificity (99.2 percent) for RA than RF does (91.6 percent), which is consistent with previous research. The results of this analysis indicate that, while ACCP assays have a higher specificity for RA, RF has a higher sensitivity. As a result, it is advised to use a mixture of ACCP and RF to diagnose rheumatoid arthritis, as this has therapeutic sensitivity close to 100%.

CONCLUSION

RA is an inflammatory condition entailing a range of biomarkers for proper individual monitoring. Some of these pens aid with the medical diagnosis, while others assess medical events and patient results. The mix of unique and standard biomarkers can help anticipate and very early diagnosis, specifically as a result of the variety of medical appearance and variance in the distribution of biomarkers in various people.

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Author's contribution

S Santheep: Manuscript preparation, Clinical Study, Literature Review
Preeti Mahawar: Guide, Manuscript editing and review
P Rosh.: Study Design

REFERENCES

1. Jiang J, Zhao M, Chang C, Wu H, Lu Q. Type i interferons in the pathogenesis and treatment of autoimmune diseases. Clinical reviews in allergy & immunology. 2020 Oct;59(2):248-72.
2. Rinaudo-Gaujous M, Blasco-Baque V, Miossec P, Gaudin P, Farge P, Roblin X, Thomas T, Paul S, Marotte H. Infliximab induced a dissociated response of severe periodontal biomarkers in rheumatoid arthritis patients. Journal of clinical medicine. 2019 May;8(5):751.
3. Cadet J, Davies KJ. Oxidative DNA damage & repair: an introduction. Free Radical Biology and Medicine. 2017 Jun 1;107:2-12.
4. Atzeni F, Talotta R, Masala IF, Bongiovanni S, Boccassini L, Sarzi-Puttini P. Biomarkers in Rheumatoid Arthritis. The Israel Medical Association Journal: IMAJ. 2017 Aug 1;19(8):512-6.
5. Lindqvist E, Eberhardt K, Bendtzen K, Heinegård D, Saxne T. Prognostic laboratory markers of joint damage in rheumatoid arthritis. Annals of the rheumatic diseases. 2005 Feb 1;64(2):196-201.
6. Ziegler A, Koch A, Krockenberger K, Großhennig A. Personalized medicine using DNA biomarkers: a review. Human genetics. 2012 Oct 1;131(10):1627-38.
7. Mathur S, Sutton J. Personalized medicine could transform healthcare. Biomedical reports. 2017 Jul 1;7(1):3-5.

Table I: Group Statistics

Parameters	RA factor	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Serum Anti-CCP (u/ml)	Negative	39	13.41	1.996	.320	<0.01**
	Positive	50	366.68	187.366	26.498	
CRP (mg/L)	Negative	39	7.108	4.6250	.7406	<0.01**
	Positive	50	21.220	22.4702	3.1778	
ESR	Negative	39	12.08	2.044	.327	<0.01**
	Positive	50	36.26	5.934	.839	

Table 2: RA Factor Variable

Variable	RA Factor	N	N*	Mean	SE Mean	StDev	Min.	Q1	Median	Q3	Max.
Age	Negative	40	0	42.95	1.82	11.52	26.00	35.25	40.00	55.00	64.00
	Positive	60	0	40.42	1.41	10.93	19.00	33.00	41.00	49.00	62.00

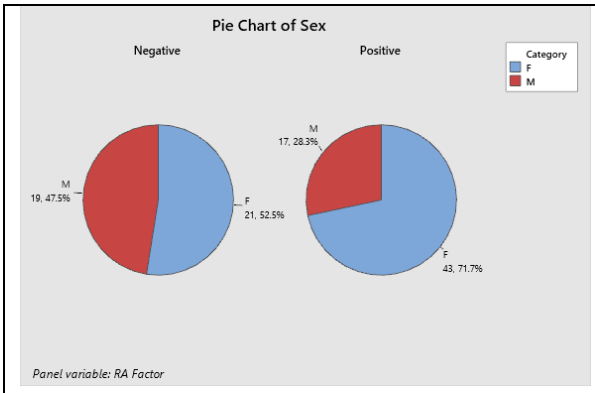

Fig.1. Pie Chart of Sex

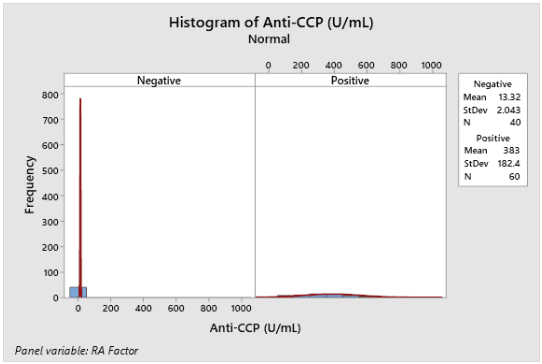

Fig.2. Histogram of Anti-CCP

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REVIEW ARTICLE

Current Review of Solid Dispersion

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ABSTRACT

Solid dispersion leads to improve the dissolution rate for poorly water soluble drugs, hence to enhance the range of bioavailability in hydrophobic drugs and etc.About10 –12% of new drug candidates(example: Diazepam, it carries sodium salicylates) are highly soluble and permeable. Maximum 60-65% of potent drug products were poorly water soluble drugs. The few aspects to be considered for the preparation of solid dispersion, such as selection of carrier and method of physicochemical characteristics. It has the general prominence consists of various types, methods, preparation and techniques involved in solid dispersion. The solvent evaporation technique with various carriers and solvent was majorly employed to prepare a solidified mass. The formulation are recently becoming more and more attractive in drug delivery for overcoming poor solubility and bioavailability issue of new drug candidates.

Keywords: solid dispersion , evaporation techniques, poor solubility, bioavailability.

INTRODUCTION

In the development of drug delivery system the scientists facing the two major problems in poor water soluble drug like solubility and dissolution.(1) Due to this solubility issues many potent drugs have low therapeutic effect but in high dose it causes the toxicity issues to the patients. (2)For this improvement in solubility and dissolution rate many of the approaches have been applied like formation of soluble salts of drugs, reduction of crystals size, conversion of drug into prodrugs, use of amorphous form, cosolvation and super disintegration, impregnating liquid drug, micronization and so on. By using this above technique we facing the many troubles like, drugs like neutral compounds and weak electrolytes are not feasible in the formation of salt. On the other hand poor wettability fine powders of hydrophobic drugs are difficult to disperse in water. (3)Solid dispersion technology is an excellent tool to

troubleshooting. This problem is enhancing the solubility, dissolution and bioavailability.(4) Solid dispersion may be defined as a group of solid product consisting of hydrophobic drug dispersed in at least one hydrophobic carrier, resulting in increased surface area and enhanced the drug solubility and dissolution rate. Generally it is dispersion of one or more active ingredient in an inert carrier or matrix at solid state. (5)Solid dispersion frequently prepared by the MELTING (fusion) method, SOLVENT method or fusion solvent method. The drug may be dispersed in the form of amorphous particles or in crystalline particles.

TYPES

FIRST CLASS OF SOLID DISPERSION

Sekiguchi and Obi was conducted the first study of SD in 1961.(11) They compare the absorption of eutectic mixture of chloramphenicol & sulfathiazole of the original formulation of the same drug. (12)Use of urea as a hydrophilic carrier results showed increased the absorption of sulfathiazole and chloramphenicol in the eutectic mixture compared to that of the conventional formulations.

SECOND CLASS OF SOLID DISPERSION

In first class of SD, it has trouble like thermodynamic instability. In second class of SD were introduced using amorphous polymeric carriers for troubleshooting instead of urea or sugar.(13) The polymeric carriers may be synthetic or natural polymer. Synthetic polymer like povidone, PEG, polymethacrylates. Natural polymer like ethyl cellulose, starch derivatives like cyclodextrins.(14) By using the PVP as carrier in ketoprofen SD increased the dissolution rate of ketoprofen compared to the conventional drug.

THIRD CLASS OF SOLID DISPERSIOND

In the preparation SD surfactant may be used alone or combined with other hydrophilic carrier. (15)In pharmaceutical industry surfactants play a crucial role to improve the solubility & BA of poorly water – soluble drug. Hydrophobicity of the drug can modify the adsorption of a surfactant, thereby reducing surface tension between two liquids or between a liquid and a solid.(16)

STRUCTURE – BASED CLASS OF SOLID DISPERSION

EUTECTIC MIXTURE

The mixture of two components that melt at a single temperature. At the eutectic point Components A and B were co-melted, where the melting point of the mixture was lower than that of component A are B alone. (17)The eutectic mixture of sulfathiazole and urea was prepared by sekaiguchi and obi. The results showed that the absorption of sulfathiazole in the eutectic mixture was improved as compared to the conventional drug.(18)

SOLID SOLUTION

Herein, the mixture of the drug and a carrier is called as SD. In the solid solution the components as continuous and discontinuous phase due to its miscibility and molecular size of solid solutions. In continuous solid solutions, the two components are mixed. Based on the bonding strength between the two components is greater that of the individual components. In discontinuous solid solutions, in solid solvent the solubility of each component is limited. Solid solutions are classified as substitution and interstitial based on molecular size. (19)

GLASS SOLUTION/GLASS SUSPENSION

The glass solution\ glass suspension are homogeneous system. In glass solution drug molecules are dissolved in glassy solvent. In glass suspension the drug molecules are suspended in a glassy solvent .For both glass solution and suspension, the glassy state is characterized by transparency and brittleness.

ADVANTAGE OF SOLID DISPERSION

- Solid dispersion results in reduction of particle size of the particles &improves the surface area and increase dissolution rate.(6)
- Increased bioavailability
- Its results in improved the bioavailability.
- SD is drug interacting with hydrophilic carries can decrease agglomeration and release in a supersaturation state, (7) resulting in rapid absorption and improved BA.
- Solid dispersion can be improve drug wettability and increase the surface area, resulting in enhanced aqueous solubility of drug.

DISADVANTAGE OF SOLID DISPERSION

- Physical instability
- Solid dispersion show change in crystalline& decreased dissolution rate with aging.(9)
- Due to the instability of Solid dispersion during the period of storage can affect drug quality and the effectiveness of treatment.(10)

METHODS INVOLVED IN SOLID DISPERSION

- Fusion method
- Solvent method
- Melting solvent method
- Supercritical fluid method
- Electro spinning method
- Solvent evaporation method
- Melt agglomeration method
- Lyophillization Techniques
- Spray-Drying method
- Dropping method solution
- Melt extrusion method
- Gel entrapment technique

FUSION METHOD

The fusion method is also known as melting method, in this method crystalline as starting materials are crystalline. (21)

ADVANTAGE

- Simplicity and economy.
- Melting under vacuum or blanket of an inert gas.

{Example: nitrogen may be employed to prevent oxidation of drug or carrier.}

DISADVANTAGE

- when drug and matrix are mix well at the heating temperature.
- phase separation can be occur

SOLVENT METHOD

In first step of a solution containing both matrix ,material and drug. The second step, solvent get evaporated result as solid dispersion (22). Using solvent method, First challenge is to mix both drug and matrix in one solution, which is difficult due to the different significantly in polarity. To minimize the drug particle size, the drug and matrix have to be dispersed in the solvent as well as possible.

ADVANTAGE

-The thermal decomposition of the drugs can be prevented.
-Because of the low temperature requires for evaporation of organic solvent.

DISADVANTAGE

-Higher cost of preparation
-Difficulty in removing liquid solvent

MELTING SOLVENT METHOD

In the initial stage drug is dissolved in a suitable liquid solvent solution and it melts the polyethylene glycol obtaining below 70◦c without removing the liquid solvent. It has been show that 5-10%(w/w) of Liquid compound could be incorporated into Polyethylene glycol 6000 without significant loss of solid property.(23)

ADVANTAGE

-Thermal decomposition of carriers can be prevented.
-Because evaporation of organic solvent has low temperature.

DISADVANTAGE

-In practical point of view, this method is limited to drugs with a low therapeutic dose.
-It is impossible the selected solvent or dissolved drug may not be miscible with the melt of polyethylene glycol.

SUPERCRITICAL FLUID METHOD

In this methods are mostly applied with carbon dioxide, is used as either a solvent for drug and matrix or as antisolvent.(24)When supercritical $C0_2$ is used as solvent matrix and drug are dissolved sprayed via the nozzle, into expansion vessel with low pressure and particles are suddenly formed. This technique does not require the use of organic solvents .Since CO_2 is consider environmentally friendly, this technique is also known as “SOLVENT FREE” and “RAPID EXPANSION OF SUPERCRITICAL SOLUTION”.

ADVANTAGE

-The supercritical anti solvents are rapidly penetrates into the particles.
-In this term process is precipitation with compressed anti oven.

DISADVANTAGE

-Organic solvents like dichloromethane or methanol has to be applied to dissolve in both drug and matrix are more in cost.

ELECTROSPINNING METHOD

Solid fibers are prepared by polymeric fluid stream solution or melt delivered through millimeter scale nozzle .In this process involves a application of a strong electrostatic field over a conductive capillary attaching to reservoir containing a polymer solution or metal and a conductive collection screen. Increasing the electrostatic field strength up, but not exceeding a critical value. A charged polymer jet is ejected from the apex of the cone.(25)The ejected charge jet is then carried to the collection screen through the electrostatic force. The columbic repulsion force is responsible for the thinning of the charged jet while its trajectory to the collection screen. The charged jet is limited by the viscosity increases, as they charged jet is dried.

ADVANTAGE

-It has tremendous potential for controlling the release of biomedicine.
- This process is simple, the cheapest.

DISADVANTAGE

-All the drugs and carriers are less economical.

SOLVENT EVAPORATION METHOD

In a volatile solvent, Drug and carriers are solubilize later it gets evaporated. The thermal decomposition of the drugs or carriers can be prevented.(26)Organic solvent evaporation occurs at low temperature. As usually the resulting films are milled and pulverized.

ADVANTAGE

- Encapsulation of hydrophobic and hydrophilic drug.
-Simple technique

DISADVANTAGE

-Cost of preparation is very high.
-Difficulty in completely removing liquid solvent.

MELT AGGLOMERATION METHOD

In this technique, used to prepare the binder that acts as a carrier. At above the melting point of the binder the drug, binder, and other excipients are heated by this method. Otherwise, dispersion of the drug sprayed on to the heated binder. Melt agglomeration method in a high shear mixer to improve the dissolution rate eg; Diazepam Solid dispersion. (27)Lactose monohydrate was used as the binder or Gelucire50/13.Binder was added by pump-on or melt in –procedures. The use of melt agglomeration resulted in a high dissolution rate at a low drug concentration.

LYOPHILLIZATION TECHNIQUES

The drug and carrier are co dissolved in a common solvent, frozen and sublimed into obtained lyophilized molecular dispersion.

SPRAY-DRYING METHOD

In this method, the drug is dissolved in a suitable solvent and required amount of carrier was dissolved in water.(28)Solutions are sonication to produce a clear solution and evaporated under vacuum. Solid dispersions are reduced in a size by sieved.

DROPPING METHOD SOLUTION

This method involves dissolving the drug and carrier in a common organic solvent and then removing the solvent by evaporation.

MELT EXTRUSION METHOD

In this method using a co-rotating twin-screw extruderis help to composed of active ingredient and carrier, and prepare by hot-stage extrusion. E.g. Sustained-release pellets.

GEL ENTRAPMENT TECHNIQUE

This is an immobilization method. Entrapment in gel may cause matrix polymerization , precipitation or coagulation. The advantages of reaction conditions usually so mild it changes in the enzyme structure occur.

PREPARATION OF SOLID DISPERSION BY SOLVENT EVAPORATION METHOD: The most commonly used in the pharmaceutical industry is the solvent evaporation method, to enhance the solubility of poorly water soluble drugs. To heat an unstable components this method has been mainly developed because the solvent is mixed by the drug and carrier instead of heat as in melting method. The carrier is used by this method with an excessive high melting point. The main principle of this method is that the volatile solvent was dissolved by both the drug and carrier under homogeneous mixing. Solid dispersion is obtained by evaporating the solvent by constant agitation.

Therefore, the solid dispersion was crushed and sieved. In 1965, Tachibana and Nakamura were first applied this method.(31) The preparation was formulated by dissolving a drug (β-carotene) and a carrier(PVP) in an organic solvent(chloroform). Then, the solvent was evaporated completely to obtain a solidified mass; it is then sieved and dried. The major advantage of this method is avoiding a degradation of drug and carrier; hence the required temperature for evaporation is low.

Crystalline drugs + hydrophobic drugs
↓
Dissolve in organic solvent
↓ removes the solvent
Solid dispersion
(Molecularly dispersed or amorphous drug in hydrophobic)

POLYMERS INVOLVED IN THE SOLID DISPERSION

POLYVINYL PYRROLIDONE (PVP)

PVP K-12
PVP K-15
PVP K-30
PVP K-60
PVP K-90
PVP K-120

COPOVIDONE

POLYETHYLENE GLYCOL (PEG)

PEG - 400
PEG -600
PEG -800
PEG-1000
PEG-1500
PEG-2000
PEG-3000
PEG-4000
PEG-6000
PEG-8000
PEG-10000
PEG-20000

HYDROXYPROPYL METHYL CELLULOSE (HPMC)

HPMC-E
HPMC-F
HPMC-K

HYDROXYPROPYL METHYLCELLULOSE ACETATESUCCINATE(HPMCAS)

HPMCAS-L
HPMCAS-M
HPMCAS-H

SOLUPLUS

SOLVENTS

The formulation of solid dispersion consists of various solvents by the following criteria:

- The drug and carrier both must be dissolved.
- After preparation the toxic solvents should be avoided due to risk of residual level. Ex: chloroform and dichloromethane. (32)
- Alternatively ethanol can be used due to its less toxic effect.
- Water based systems are preferred.
- Care must be taken for surfactants to create carrier drug solution, or else they can reduce the glass transition temperature.

CLASS I SOLVENT (SOLVENTS TO BE AVOIDED)

Solvents involved in this class may produce deleterious environmental effects, So it must not be taken into use.

CLASS II SOLVENTS (SOLVENTS TO BE LIMITED)

Due to their inherent toxicity, the solvent must be used limitedly in pharmaceutical products

CLASS III SOLVENTS (SOLVENT WITH LOW TOXIC POTENT)

These class III solvents are less toxic and low risk in human health.

CLASS IV SOLVENTS

These class IV solvent may interested to do manufactures of excipients, drug substance or products.

EVALUATION OF SOLID DISPERSION

- SOLUBILITY STUDIES
- DRUG CONTENT
- BULK DENSITY
- TAPPED DENSITY
- ANGLE OF REPOSE
- *IN-VITRO* RELEASE STUDIES

SOLUBILITY STUDIES

Solubility measurement were performed according to the method (36).The solid dispersion sample was taken 100mg and mixed with 10ml of distilled water in Stoppard conical flask in an orbital shaker for 24hrs at room temperature and the solution was filtrated through the filtered paper and the filtrated solution was diluted properly mix with the suitable buffer and the solution is analyzed in UV.

DRUG CONTENT

Solid dispersion equivalent sample were weighted accurately and dissolved in given solution. And the solution was filtered, (37)diluted suitably and the drug content was analyzed by UV spectrophotometer, the actual drug content was calculated using following;

% Drug content = (Actual amount of drug in solid dispersion / theoretical amount of drug in solid dispersion) x 100

DETERMINATION OF FLOW OF PROPERTIES

BULK DENSITY

Weigh above 10grm of given powder and transfer the powder into the Nessler cylinder and tapped the Nessler cylinder approximately 100 times to the get appropriates concordant values.

Bulk density = mass of the powder/ Bulk volume

TAPPED DENSITY

Weigh the powder and transfer into the measuring cylinder and place cylinder in a tapping apparatus and perform the tapping note down the initial volume and tapped volume and calculate the tapped density.(38)

Tapped density = Mass of the powder / Tapped volume

Carr's index and Hausner'sratio

These ratios are calculated by using the following formula.

Carr's index = (tapped density – Bulk density)/ Tapped density)x 100

Hausners ratio = tapped density / bulk density

ANGLE OF REPOSE

Funnel is fixed at the particular height in 6 to12 cm in the burette stand. Graph paper placed below the funnel on the table and he given powdered drug whose angle of repose is to be determined and slowly pass through the funnel until it found pill and care the is taken see the drug particle split and shows the single of funnel. (39)Further addition of the drug is stopped as soon as the pill successfully bottom of the funnel. The circumstance of the pill is drawn without disturbing the pill .radius of the pill note down as R. the angle the repose is calculated

$\theta = \tan^{-1} h/r$

The experiment is repeated when the funnel is fixed at the different height.

***IN-VITRO* RELEASE STUDIES**

In vitro dissolution studies were performed for prepared solid dispersion.(40) The conditions were maintained for the dissolution process;

Instrument

LABINDIA DS-8000 Dissolution test apparatus, paddle type apparatus, temperature up to the given, RPM, Dissolution Medium, volume of medium, sampling intervals and sample volumes are taken.

APPLICATION OF SOLID DISPERSION

- To increase the drug absorbtion, solubility, bioavailability, dissolution rate.(41)
- Enchanement of solubility and stability.
- Formulation of fast released dosage form.
- To decrease the adverse effect of certain drugs.
- Masking of unpleasant taste and smell of drugs.
- Development of drug release from ointment and creams..
- The liquid or gaseous compounds are transformed into a solid dosage form.

RESULT AND DISCUSSION

Solid dispersion formulations are recently play a major role in drug delivery system for overcoming poor solubility and bioavailability issues of new drug candidates, but their commercial application is limited. The various methods have been tried recently to overcome the limitation and make the preparation practically feasible. The various issues that impeded the commercial development of solid dispersions includes;

- Inability to scale bench top formulation to manufacturing sized batches,
- Difficulty to control physicochemical properties
- Difficulty in delivery solid dispersion formulation as tablet or capsule dosage form.
- Physical and chemical instability of the drug and\or formulation itself.

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REFERENCES

1. Shewach, D.S.; Kuchta, R.D. Introduction to cancer chemotherapeutics. Chem. Rev. 2009, 109, 2859–2861. [CrossRef] [PubMed]
2. Hwang, H.S.; Shin, H.; Han, J.; Na, K. Combination of photodynamic therapy (PDT) and anti-tumor immunity in cancer therapy. J. Pharm. Investig. 2018, 48, 143–151. [CrossRef]
3. Arias, J.L. Drug targeting strategies in cancer treatment: An overview. Mini Rev. Med. Chem. 2011, 11, 1–17. [CrossRef] [PubMed]
4. Lin, H.-M.; Lin, H.-Y.; Chan, M.-H. Preparation, characterization, and in vitro evaluation of folate-modified mesoporous bioactive glass for targeted anticancer drug carriers. J. Mater. Chem. B 2013, 1, 6147. [CrossRef]
5. Choi, J.-S.; Kwon, S.-H.; Lee, S.-E.; Jang, W.S.; Byeon, J.C.; Jeong, H.M.; Park, J.-S. Use of acidifier and solubilizer in tadalafil solid dispersion to enhance the in vitro dissolution and oral bioavailability in rats. Int. J. Pharm. 2017, 526, 77–87. [CrossRef] [PubMed]
6. Mohammadian, M.; Salami, M.; Momen, S.; Alavi, F.; Emam-Djomeh, Z.; Moosavi-Movahedi, A.A. Enhancing the aqueous solubility of curcumin at acidic condition through the complexation with whey protein nanofibrils. Food Hydrocoll. 2019, 87, 902–914. [CrossRef]
7. Chen, X.; McClements, D.J.; Zhu, Y.; Chen, Y.; Zou, L.; Liu, W.; Cheng, C.; Fu, D.; Liu, C. Enhancement of the solubility, stability and bioaccessibility of quercetin using protein-based excipient emulsions. Food Res. Int. 2018, 114, 30–37. [CrossRef] [PubMed]
8. Kim, C.H.; Lee, S.G.; Kang, M.J.; Lee, S.; Choi, Y.W. Surface modification of lipid-based nanocarriers for cancer cell-specific drug targeting. J. Pharm. Investig. 2017, 47, 203–227. [CrossRef]
9. Zhao, Y.; Xie, X.; Zhao, Y.; Gao, Y.; Cai, C.; Zhang, Q.; Ding, Z.; Fan, Z.; Zhang, H.; Liu, M.; et al. Effect of plasticizers on manufacturing ritonavir/copovidone solid dispersions via hot-melt extrusion: Preformulation, physicochemical characterization, and pharmacokinetics in rats. Eur. J. Pharm. Sci. 2019, 127, 60–70. [CrossRef]
10. Smeets, A.; Koekoekx, R.; Clasen, C.; Van den Mooter, G. Amorphous solid dispersions of darunavir: Comparison between spray drying and electrospraying. Eur. J. Pharm. Biopharm. 2018, 130, 96–107. [CrossRef]
11. Sekiguchi, K.; Obi, N. Studies on absorption of eutectic mixture. I. A comparison of the behavior of eutectic mixture of sulfathiazole and that of ordinary sulfathiazole in man. Chem. Pharm. Bull. (Tokyo) 1961, 9, 866–872. [CrossRef]
12. Sekiguchi, K.; Obi, N.; Ueda, Y. Studies on absorption of eutectic mixture. II. Absorption of fused conglomerates of chloramphenicol and ure in rabbits. Chem. Pharm. Bull. (Tokyo) 1964, 12, 134–144. [CrossRef]
13. Levy, G. Effect of particle size on dissolution and gastrointestinal absorption rates of pharmaceuticals. Am. J. Pharm. Sci. Support. Public Health 1963, 135, 78–92.
14. Kanig, J.L. Properties of fused mannitol in compressed tablets. J. Pharm. Sci. 1964, 53, 188–192. [CrossRef] 51. Eloy, J.O.; Marchetti, J.M. Solid dispersions containing ursolic acid in poloxamer 407 and PEG 6000: A comparative study of fusion and solvent methods. Powder Technol. 2014, 253, 98–106. [CrossRef]
15. Otto, D.P.; Otto, A.; de Villiers, M.M. Experimental and mesoscale computational dynamics studies of the relationship between solubility and release of quercetin from PEG solid dispersions. Int. J. Pharm. 2013, 456, 282–292. [CrossRef] [PubMed]

16. Reginald-Opara, J.N.; Attama, A.; Ofokansi, K.; Umeyor, C.; Kenechukwu, F. Molecular interaction between glimepiride and soluplus ®-PEG 4000 hybrid based solid dispersions: Characterisation and anti-diabetic studies. Int. J. Pharm. 2015, 496, 741–750. [CrossRef]
17. Jiménez de los Santos, C.J.; Pérez-Martínez, J.I.; Gómez-Pantoja, M.E.; Moyano, J.R. Enhancement of albendazole dissolution properties using solid dispersions with Gelucire 50/13 and PEG 15000. J. Drug Deliv. Sci. Technol. 2017, 42, 261–272. [CrossRef]
18. Damian, F.; Blaton, N.; Naesens, L.; Balzarini, J.; Kinget, R.; Augustijns, P.; Van den Mooter, G. Physicochemical characterization of solid dispersions of the antiviral agent UC-781 with polyethylene glycol 6000 and Gelucire 44/14. Eur. J. Pharm. Sci. 2000, 10, 311–322. [CrossRef]
19. Li, F.; Hu, J.; Deng, J.; Su, H.; Xu, S.; Liu, J. In vitro controlled release of sodium ferulate from compritol 888 ATO-based matrix tablets. Int. J. Pharm. 2006, 324, 152–157. [CrossRef]
20. Panda, T.; Das, D.; Panigrahi, L. Formulation development of solid dispersions of bosentan using Gelucire 50/13 and poloxamer 188. J. Appl. Pharm. Sci. 2016, 6, 027–033. [CrossRef]
21. Lloyd GR, A calorimetric investigation into the interaction between paracetamol and polyethlene glycol 4000 in physical mixes and solid dispersions, Eur. J. Pharm. Biopharm. 2003; 48: 59–65.
22. Rodier E, A three step supercritical process to improve the dissolution rate of Eflucimibe, Eur. J. Pharm. Sci. 2005; 26: 184–193.
23. Won DH, Improved physicochemical characteristics of felodipine solid dispersion particles by supercritical antisolvent precipitation process, Int. J. Pharm. 2005; 301: 199– 208.
24. Yoshihashi Y, Estimation of physical stability of amorphous solid dispersion us4. Tiwari Ruchi, Tiwari Gaurav, Srivastava Birendra, Rail K Awani, Singh Pranveer, Solid Dispersions: An Overview To Modify Bioavailability Of Poorly Water Soluble Drugs, International Journal of PharmTech Research, 2009, Vol.1, No.4, 1338-1349.
25. Chaulang G, Patil K, Ghodke D, Khan S, Yeole P, Preparation and Characterization of Solid Dispersion Tablet of Furosemide with Crospovidone, Research J Pharm and Tech. 2008, 1(4), 386-389.
26. Sharma Anshu, Jain C P, Solid dispersion: A promising technique to enhance solubility of poorly water soluble drug , Internati100. Alves, L.D.S.; de La Roca Soares, M.F.; de Albuquerque, C.T.; da Silva, É.R.; Vieira, A.C.C.; Fontes, D.A.F.; Figueirêdo, C.B.M.; SoaresSobrinho, J.L.; RolimNeto, P.J. Solid dispersion of efavirenz in PVP K-30 by conventional solvent and kneading methods. Carbohydr. Polym. 2014, 104, 166–174. [CrossRef]
27. Yin, L.-F.; Huang, S.-J.; Zhu, C.-L.; Zhang, S.-H.; Zhang, Q.; Chen, X.-J.; Liu, Q.-W. In vitro and in vivo studies on a novel solid dispersion of repaglinide using polyvinylpyrrolidone as the carrier. Drug Dev. Ind. Pharm. 2012, 38, 1371–1380. [CrossRef]
28. Nguyen, M.N.-U.; Van Vo, T.; Tran, P.H.-L.; Tran, T.T.-D. Zein-based solid dispersion for potential application in targeted delivery. J. Pharm. Investig. 2017, 47, 357–364. [CrossRef]
29. Journal of Drug Delivery, 2011,3, 149-170.ing differential scanning calorimetry, J. Therm. Anal. Calorim. 2006; 85: 689– 69292. Shuai, S.; Yue, S.; Huang, Q.; Wang, W.; Yang, J.; Lan, K.; Ye, L. Preparation, characterization and in vitro/vivo evaluation of tectorigenin solid dispersion with improved dissolution and bioavailability. Eur. J. Drug Metab. Pharmacokinet. 2016, 41, 413–422. [CrossRef]
30. Daravath, B.; Tadikonda, R.R.; Vemula, S.K. Formulation and pharmacokinetics of Gelucire solid dispersions of flurbiprofen.
31. Drug Dev. Ind. Pharm. 2015, 41, 1254–1262. [CrossRef]
32. Mustapha, O.; Kim, K.S.; Shafique, S.; Kim, D.S.; Jin, S.G.; Seo, Y.G.; Youn, Y.S.; Oh, K.T.; Yong, C.S.; Kim, J.O.; et al. Comparison of three different types of cilostazol-loaded solid dispersion: Physicochemical characterization and pharmacokinetics in rats. Colloids Surfaces B Biointerfaces 2017, 154, 89–95. [CrossRef]
33. Kim, S.-J.; Lee, H.-K.; Na, Y.-G.; Bang, K.-H.; Lee, H.-J.; Wang, M.; Huh, H.-W.; Cho, C.-W. A novel composition of ticagrelor by solid dispersion technique for increasing solubility and intestinal permeability. Int. J. Pharm. 2019, 555, 11–18. [CrossRef] [PubMed]
34. Al-Hamidi, H.; Obeidat, W.M.; Nokhodchi, A. The dissolution enhancement of piroxicam in its physical mixtures and solid dispersion formulations using gluconolactone and glucosamine hydrochloride as potential carriers. Pharm. Dev. Technol. 2015, 20, 74–83. [CrossRef] [PubMed]

35. Zhang, W.; Zhang, C.; He, Y.; Duan, B.; Yang, G.; Ma, W.; Zhang, Y. Factors affecting the dissolution of indomethacin solid dispersions. AAPS PharmSciTech 2017, 18, 3258–3273. [CrossRef]
36. Hasegawa S: Effects of water content in physical mixture and heating temperature on crystallinity of troglitazone-PVP K30 solid dispersions prepared by closed melting method. International Journal of Pharmaceutics 2003; 302(29): 103–112.
37. Rodier E: A three step supercritical process to improve the dissolution rate of Eflucimibe. European Journal of Pharmaceutical Sciences 2005; 26: 184–193.
38. Won DH: Improved physicochemical characteristics of felodipine solid dispersion particles by supercritical antisolvent precipitation process. International Journal of Pharmaceutics 2005; 301: 199–208.
39. Lloyd GR: A calorimetric investigation into the interaction between paracetamol and polyethlene glycol 4000 in physical mixes and solid dispersions. European Journal of Pharmaceutics and Biopharmaceutics 2003; 48: 59–65.
40. Yoshihashi Y: Estimation of physical stability of amorphous solid dispersion using differential scanning calorimetry. Journal of Thermal Analysis and Calorimetric 2006; 85: 689– 692.
41. M Vasanthavada; WQ Tong; Y Joshi; MS Kislalioglu. Pharm Res., 2004, 21(9), 1598-1606.

Table 1. Current Drug Available in Market

PRODUCT	DRUG	DOSAGE FORM	POLYMER USED
Gris-PEG	Griseofulvin	Tablet	PEG
Intelence	Etravirine	Tablet	-
Isoptin	Verapamil hydrochloride	Tablet	MCC, Hypromellose
Kaletra	Lopinavir/Ritonavir	Capsule	PVP
Nivadil	Nivaldipine	Tablet	HPMC
Pro-Gral	Tacrolimus	Capsule and injection	HPMC, croscarmellosesodium, hypromellose
Sporanox	Itraconazole	Capsule and Oral Solution	HPMC &PEG

Table 2. Class I Solvent (Solvents To Be Avoided)

SOLVENT	CONCENTRATION LIMIT (PPM)	EFFECT
Benzene	2	-
Carbon tetrachloride	4	Carcinogenic, toxic, environmental hazards
1,2-dichloroethane	5	Toxic
1,1-dichloroethane	8	Toxic
1,1,1-trichloroethane	1500	Environmental hazards

Table 3. Class II Solvents (Solvents To Be Limited)

SOLVENT	PERMITTED DAILY EXPOSURE (mg/day)	CONCENTRATION LIMIT (ppm)
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-dichloroethene	18.7	1870
Ethylene glycol	6.2	620
Methanol	30.0	3000
Pyridine	2.0	200
Toluene	8.9	890

Table 4. Class III Solvents (Solvent with Low Toxic Potent)

\ Acetic acid	Ethyl ether
1-butanol	Formic acid
2-butanol	Heptane
Acetone	Isobutylacetate
Butyl acetate	Isopropylacetate
Dimethyl sulfoxide	Methyl acetate
Ethanol	3-methyl-1-butanol
Ethyl acetate	Pentane
1-propanol	1-pentanol
2-propanol	Propylacetate

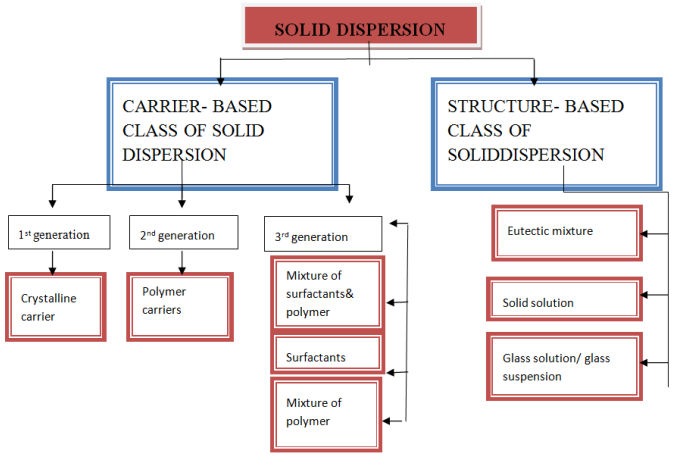

Fig.1. Types

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RESEARCH ARTICLE

$\delta\hat{\hat{g}}$-Continuity and it's Decompositions

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ABSTRACT

We make this paper the concepts of decomposition of $\boldsymbol{\delta\hat{\hat{g}}}$-continuity in topological spaces. More over, we deal the concepts of $\boldsymbol{\delta\hat{\hat{g}}}$lc*-set and $\boldsymbol{\delta\hat{\hat{g}}}$lc*-continuous functions by using $\boldsymbol{\delta\hat{\hat{g}}}$-continuity, prove that a function is $\boldsymbol{\delta}$-continuous if and only if it is both $\boldsymbol{\delta\hat{\hat{g}}}$-continuous and $\boldsymbol{\delta\hat{\hat{g}}}$lc*-continuous. Further, fresh scheme such $T_{\delta\hat{\hat{g}}}$-spaces and ${}_gT_{\delta\hat{\hat{g}}}$-spaces. Using these spaces we obtain one more decomposition of $T_{1/2}$-spaces.
2010 Mathematics Subject Classification: 54A05, 54A10, 54C08, 54C10

Keywords: $\delta\hat{\hat{g}}$lc*-set, $\delta\hat{\hat{g}}$lc*-continuous functions and $\delta\hat{\hat{g}}$-continuous functions and $\delta\hat{\hat{g}}$-irresolute functions.

INTRODUCTION

Levine [10, 11] was set up the ideas such as semi-open sets and generalized closed sets (for a short time g-closed set) are investigated its fundamental properties. This concept was shown to be creative and exceedingly useful. Velicko [17] was introduced by δ-closed sets and it is well known that the collection of all δ-closed sets of a topological space forms a topology and is denote by τ_δ. Dontchev and Maki [8] were pioneer the idea of δ-generalized closed sets in topological spaces. In the field of canvassers studied and investigated for some examples [1, 2, 4, 5, 6, 12] are given. We make this paper the concepts of decomposition of $\delta\hat{\hat{g}}$-continuity in topological spaces. More over, we deal the concepts of $\delta\hat{\hat{g}}$lc*-set and $\delta\hat{\hat{g}}$lc*-continuous functions by using $\delta\hat{\hat{g}}$-continuity, prove that a function is δ-continuous if and only if it is both $\delta\hat{\hat{g}}$-continuous and $\delta\hat{\hat{g}}$lc*-continuous. Further, new scheme called $T_{\delta\hat{\hat{g}}}$-spaces and ${}_gT_{\delta\hat{\hat{g}}}$-spaces. Using these spaces we obtain a new decomposition of $T_{1/2}$-spaces.

Preliminaries

Right through this paper (X, τ) and (Y, σ) (or X and Y) represent topological spaces on which no separation axioms are assumed unless otherwise mentioned. We recall some basic definitions which are useful in the sequel.

Definition 2.1 A subset A of a space (X, τ) is called

1. semi-open set [11] if A ⊆ cl(int(A)).
2. regular open set [16] if A = int(cl(A)).

The complements of the above mentioned open sets are called their respective closed sets.

Definition 2.2 *[17]* A point x of a space X is called a δ-adherent point of a subset A of X if int(cl(U)) ∩ A ≠ ∅, for every open set U containing x. The set of all δ-adherent points of A is called the δ-closure of A and is denoted by cl $_\delta$(A). A subset A of a space X is called δ-closed if and only if A = cl $_\delta$(A). The complement of a δ-closed set is called δ-open.

Definition 2.3 *A* subset A of a space (X, τ) is called

1. a generalized closed set (briefly, g-closed) set [10] if cl(A) ⊆ U whenever A ⊆ U and U is open.
2. a semi-generalized closed set (briefly, sg-closed) set [4] if scl(A) ⊆ U whenever A ⊆ U and U is semi-open.
3. a $\hat{g}$-closed set [9] if cl(A) ⊆ U whenever A ⊆ U and U is sg-open.
4. a δ-generalized closed (briefly, δg-closed) set [8] if cl $_\delta$(A) ⊆ U whenever A ⊆ U and U is open.
5. a $\delta\hat{g}$-closed set [3] if $cl_\delta(A) \subseteq U$ whenever $A \subseteq U$ and U is sg-open.

The complements of the above mentioned closed sets are called their respective open sets.

Proposition 2.4 [3] In a space (X, τ), every δ-closed set is $\delta\hat{g}$-closed.

Proposition 2.5 [3] In a space (X, τ), every $\delta\hat{g}$-closed set is g-closed.

Proposition 2.6 [3] If a set A is $\delta\hat{g}$-closed in (X, τ) then cl_δ(A) − A contains no nonempty sg-closed set in (X, τ).

Lemma 2.7 [8] In any space, a singleton is δ-open if and only if it is regular open.

Decomposition of δ-continuity

Definition 3.1 A subset A of a space (X, τ) is said to be a $\delta\hat{g}$lc*-set if I = M ∩ T, where M is sg-open and T is δ-closed.

Proposition 3.2 In a space (X, τ), any δ-closed ⇒ $\delta\hat{g}$lc*-set.

Remark 3.3 Reverse implication of the Proposition 3.2 is not true as shown in the following example.

Example 3.4 Let X = {1,2,3} with τ = {ϕ, {2}, X}. In the space (X, τ), then {2,3} is a $\delta\hat{g}$lc*-set but not δ-closed.

Remark 3.5 In a space (X, τ), the following case shows that the idea of $\delta\hat{g}$-closed sets and the idea of $\delta\hat{g}$lc*-sets are independent.

Example 3.6 1. Let X = {1,2,3} with τ = {ϕ, {1,2}, X}. Then {1,3} is $\delta\hat{g}$-closed set but not $\delta\hat{g}$lc*-set in (X, τ).

2. In Example 3.4, then {1,2} is $\delta\hat{g}$lc*-set but not $\delta\hat{g}$-closed set in (X, τ).

Theorem 3.7 Let (X, τ) be a topological space. Then a subset A of (X, τ) is δ-closed if and only if it is both $\delta\hat{g}$-closed and $\delta\hat{g}$lc*-set.

Proof. Obviously. Assuming that I is both $\delta\hat{g}$-closed and $\delta\hat{g}$lc*-set. Then I = S ∩ K, where S is sg-open and K is δ-closed in (X, τ). Therefore, I ⊆ S and I ⊆ K and so by hypothesis, cl $_\delta$(I) ⊆ S and cl $_\delta$(I) ⊆ K. Thus cl $_\delta$(I) ⊆ S ∩ K = I and hence cl $_\delta$(I) = I i.e., I is δ-closed in (X, τ).

Definition 3.8 A function f : (X, τ) → (Y, σ) is called a

1. $\delta\hat{g}$lc*-continuous if for any closed set K of (Y, σ), f $^{-1}$(K) is a $\delta\hat{g}$lc*-set.
2. $\delta\hat{g}$-continuous if for any closed set K of (Y, σ), f $^{-1}$(K) is $\delta\hat{g}$-closed.

Proposition 3.9 Each δ-continuous function ⇒ $\delta\hat{g}$-continuous.

Proof. It follows from Proposition 2.4(1).

Remark 3.10 The following example show that reverse implications of Proposition 3.9 is not true.

Example 3.11 Let X = Y = {1,2,3} with $\tau = \{\Phi, \{1,2\}, X\}$ and $\sigma = \{\Phi, \{2\}, Y\}$. We have $\delta C(X) = \{\Phi, X\}$ and $\delta\hat{\hat{G}}C(X) = \{\Phi, \{3\}, \{1,3\}, \{2,3\}, X\}$. Let $f: (X,\tau) \to (Y,\sigma)$ be the identity function. Then f is $\delta\hat{g}$-continuous but not δ-continuous, since $f^{-1}(\{1,3\}) = \{1,3\}$ is not δ-closed in (X, τ).

Remark 3.12 The following example show that every δ-continuous function is$\delta\hat{g}$lc*-continuous but not converse.

Example 3.13 Let X = Y = {1,2,3} with $\tau = \{\Phi, \{2\}, X\}$ and $\sigma = \{\Phi, \{1\}, \{1,3\}, Y\}$. Let $f: (X,\tau) \to (Y,\sigma)$ be the identity function. Then f is $\delta\hat{g}$lc*-continuous function but not δ-continuous, since for the closed set {2} in (Y, σ), $f^{-1}(\{2\}) = \{2\}$, which is not δ-closed in (X, τ).

Remark 3.14 The following case shows that $\delta\hat{g}$-continuity and $\delta\hat{g}$lc*-continuity are independent.

Example 3.15 1. Let X = Y = {1,2,3} with $\tau = \{\Phi, \{1,2\}, X\}$ and $\sigma = \{\Phi, \{2\}, Y\}$. We have $f: (X,\tau) \to (Y,\sigma)$ be the identity function. Then f is $\delta\hat{g}$-continuous but not $\delta\hat{g}$lc $*$-continuous.

2. Let X = Y = {1,2,3} with $\tau = \{\Phi, \{1\}, X\}$ and $\sigma = \{\Phi, \{2,3\}, Y\}$. Let $\sigma = \{\Phi, \{2\}, Y\}$ be the identity function. Then f is $\delta\hat{g}$lc $*$-continuous but not $\delta\hat{g}$-continuous.

Theorem 3.16 A function $f: (X,\tau) \to (Y,\sigma)$ is δ-continuous if and only if it is both $\delta\hat{g}$-continuous and $\delta\hat{g}$lc $*$-continuous.

Proof. Assuming that f is δ-continuous. It is follows from Proposition 3.9 and Remark 3.14, then f is both $\delta\hat{g}$-continuous and $\delta\hat{g}$lc $*$-continuous.

Conversely, assume that f is both $\delta\hat{g}$-continuous and $\delta\hat{g}lc*$-continuous. Let K be a closed subset of (Y, σ). Then $f^{-1}(K)$ is both $\delta\hat{g}$-closed and $\delta\hat{g}lc*$-set. It is follows from Theorem 3.7, we have $f^{-1}(K)$ is a δ-closed set in (X, τ) and so f is δ-continuous.

Decomposition of $T_{1/2}$-spaces

Definition 4.1 A space (X, τ) is said to be a $T_{\delta\hat{g}}$-space if every $\delta\hat{g}$-closed set in it is δ-closed.

Example 4.2 1. Let X = {1,2,3} with $\tau = \{\Phi, \{1\}, \{2,3\}, X\}$. We have $\delta\hat{\hat{G}}C(X) = \{\Phi, \{1\}, \{2,3\}, X\}$. Then (X, τ) is a $T_{\delta\hat{g}}$-space.

2. Let X = {1,2,3} with $\tau = \{\Phi, \{1\}, X\}$. Then (X, τ) is not a $T_{\delta\hat{g}}$-space.

Theorem 4.3 For a topological space (X, τ), the following statements are equivalent.

1. (X, τ) is a $T_{\delta\hat{g}}$-space.
2. each singleton of (X, τ) is either δ-open or sg-closed.
3. each singleton of (X, τ) is either regular open or sg-closed.

Proof. (1) ⇒ (2). If {x} is not sg-closed, then $X - \{x\}$ is not sg-open. Hence X is only sg-open set containing $X - \{x\}$. Therefore $cl_\delta(X - \{x\}) \subseteq X$. Thus $X - \{x\}$ is $\delta\hat{g}$-closed. By (i) $X - \{x\}$ is δ-closed, i.e. {x} is δ-open.

(2) ⇒ (1). Let $A \subseteq X$ be $\delta\hat{g}$-closed. Let $x \in cl_\delta(A)$. We consider the following two cases:

Case (a) Let {x} be sg-open. Since x belongs to the δ-closure of A, then $\{x\} \cap A \neq \Phi$. This shows that $x \in A$.

Case (b) Let {x} be sg-closed. If we assume that $x \notin A$, then we would have $x \in cl_\delta(A) - A$ which cannot happen according to Proposition 2.6. Hence $x \in A$. So in both cases we have $cl_\delta(A) \subseteq A$. Since the reverse inclusion is trivial, then $A = cl_\delta(A)$ or equivalently A is δ-closed.

(2) ⇔ (3) It follows from Lemma 2.7.

Definition 4.4 A space X is called ${}_gT_{\delta\hat{g}}$-space if every g-closed set in it is $\delta\hat{g}$-closed.

Example 4.5 1. Let X = {1,2,3} with $\tau = \{\Phi, \{1,2\}, X\}$. We have $GC(X) = \delta\hat{\hat{G}}C(X) = \{\Phi, \{3\}, \{1,3\}, \{2,3\}, X\}$. Then (X, τ) is a ${}_gT_{\delta\hat{g}}$-space.

2. In Example 4.2(2), we have $GC(X) = \{\Phi, \{2\}, \{3\}, \{1,2\}, \{1,3\}, \{2,3\}, X\}$ and $\delta\hat{\hat{G}}C(X) = \{\Phi, \{2,3\}, X\}$. Then (X, τ) is not a ${}_gT_{\delta\hat{g}}$-space.

Proposition 4.6 Every semi-regular $T_{1/2}$-space is $T_{\delta\hat{g}}$-space.

Proof. Follows from Proposition 2.5.

Remark 4.7 The following example show that converse of Proposition 4.6 is not true.

Example 4.8 In Example 4.2(1), we have GC(X) = ρ(X). Hence (X, τ) is $T_{\delta\hat{g}}$-space but it is not a $T_{1/2}$-space.

Proposition 4.9 Any semi-regular $T_{1/2}$-space ⇒ ${}_gT_{\delta\hat{g}}$-space.

Proof. Obvious.

Remark 4.10 The following example show that reverse implication of Proposition 4.9 is not true.

Example 4.11 In Example 3.6(1). We have GC(X) = $\delta\hat{\hat{G}}$C(X) = {Φ, {3}, {1,3}, {2,3}, τ}. Hence (X, τ) is a ${}_gT_{\delta\hat{g}}$ -space but not a $T_{1/2}$-space.

Remark 4.12 The following case show that idea of $T_{\delta\hat{g}}$-spaces and the idea of ${}_gT_{\delta\hat{g}}$-spaces are independent.

Example 4.13 1. In Example 3.6(1). We have GC(X) = {Φ, {3}, {1,3}, {2,3}, X} and δC(X) = {Φ, X}. Then (X, τ) is a ${}_gT_{\delta\hat{g}}$-space but it is not a $T_{\delta\hat{g}}$-space.

2. In Example 4.2(1). Then (X, τ) is a $T_{\delta\hat{g}}$-space but it is not a ${}_gT_{\delta\hat{g}}$-space.

Theorem 4.14 A semi-regular space (X, τ) is $T_{1/2}$ if and only if it is both $T_{\delta\hat{g}}$-space and ${}_gT_{\delta\hat{g}}$-space.

Proof. Follows from Propositions 4.6 and 4.9.

Assuming that (X, τ) is both $T_{\delta\hat{g}}$-space and ${}_gT_{\delta\hat{g}}$-space. Let A be a g-closed set of (X, τ). Then A is δĝ-closed, since (X, τ) is ${}_gT_{\delta\hat{g}}$-space. Again since (X, τ) is a $T_{\delta\hat{g}}$-space, A is δ-closed set in (X, τ) and so (X, τ) is $T_{1/2}$.

$\delta\hat{g}$-Irresolute functions

Definition 5.1 A function f : X → Y is called δĝ-irresolute if f^{-1}(H) is δĝ-closed in X for every δĝ-closed set H of Y.

Theorem 5.2 Let X be semi-regular. Then for a function f : X → Y, the following statements are equivalent.

1. f is δĝ-continuous.
2. f is ĝ-continuous.

Theorem 5.3 Let f : X → Y and g : Y → Z be two functions. Then

1. g o f is δĝ-continuous, if g is continuous and f is δĝ-continuous.
2. g o f is δĝ-irresolute, if g is δĝ-irresolute and f is δĝ-irresolute.
3. g o f is δĝ-continuous, if g is δĝ-continuous and f is δĝ-irresolute.
4. Let Y be $T_{\delta\hat{g}}$-space. Then g o f is δĝ-continuous, if g is δĝ-continuous and f is δĝ-continuous.
5. Let Y be a semi-regular space. Then g o f is δĝ-continuous, if g is ĝ-continuous and f is δĝ-irresolute.

REFERENCES

1. Andrijevic, D.: Semi-preopen sets, Mat. Vesnik, 38(1986), 24-32.
2. Arya, S. P. and Nour, T. M.: Characterizations of s-normal spaces, Indian J. Pure Appl. Math., 21(8)(1990), 717-719.
3. Balasubramaniyan, K and Sridharan, K.: $\delta\hat{g}$–Closed sets in topological spaces (To appear).
4. Bhattacharyya, P. and Lahiri, B. K.: Semi-generalized closed sets in topology, Indian J. Math., 29(3)(1987), 375-382.
5. Di Maio, G. and Noiri, T.: On s-closed spaces, Indian J. Pure Appl. Math., 18(3)(1987), 226-233.
6. Dlaska, K. and Ganster, M.: S-sets and co-S-closed topologies, Indian J. Pure Appl. Math., 23(1992), 731-737.
7. Dontchev, J.: On generalizing semi-preopen sets, Mem. Fac. Sci. Kochi Univ. Ser. A. Math.,16(1995), 35-48.
8. Dontchev, J. and Ganster, M.: On δ-generalized closed sets and $T_{3/4}$-spaces, Mem. Fac. Sci.Kochi Univ. (Math)., 17(1996), 15-31.
9. Garg, M., Agarwal, S., and Goal, C. K.: On $\hat{g}$-closed sets in topological spaces, Acta Ciencia Indica, XXXIII(4)M(2007), 1643-1652.
10. Levine, N.: Generalized closed sets in topology, Rend. Circ. Math. Palermo, 19(2)(1970), 89-96.
11. Levine, N.: Semi-open sets and semi-continuity in topological spaces, Amer. Math. Monthly, 70(1963), 36-41.

12. Mashhour, A. S., Abd El-Monsef, M. E. and El-Deeb, S. N.: On precontinuous and weak pre continuous mappings, Proc. Math. and Phys. Soc. Egypt, 53(1982), 47-53.
13. Njastad, O.: On some classes of nearly open sets, Pacific J. Math., 15(1965), 961-970.
14. Noiri, T., Maki, H. and Umehara, J.: Generalized preclosed functions, Mem. Fac. Sci. Kochi Univ. Math., 19(1998), 13-20.
15. Noiri, T.: On δ-continuous functions, J. Korean. Math. Soc., 18(1980), 161-166.
16. Stone, M. H.: Application of the theory of Boolean rings to general topology, Trans. Amer. Math. Soc., 41(1937), 375-381.
17. Velicko, N. V.: H-closed topological spaces, Amer. Math. Soc. Transl., 78(1968), 103-118.

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RESEARCH ARTICLE

Evaluation of New Stability Indicating Method for the Determination of Flupirtine and Thiocolchicoside in Pharmaceutical Dosage Form By RP-HPLC Method

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the estimation of Flupirtine and Thiocolchicoside in pharmaceutical dosage form. The column used was Phenomenex luna C18(250×4.6mm)5µ, in isocratic mode, with mobile phase containing Phosphate buffer and Acetonitrile (65:35v/v), pH was adjusted to 5, with a flow rate of 1ml/min, and effluents were monitored at UV 260nm. The retention times of Flupirtine and Thiocolchicoside were 4.3 min and 8.3.min respectively. The linearity for Flupirtine and Thiocolchicoside were in the range of 50-250 µg/ml and 4-20 µg/ml respectively with correlation coefficient of r2=0.9999 for both the drugs. The recoveries of Flupirtine and Thiocolchicoside maleate were found to be 98.5% and 97.84% respectively. The % RSD from reproducibility was found to be <2%. The proposed method was statistically evaluated and can be applied for routine quality control analysis of Flupirtine and Thiocolchicoside in pharmaceutical dosage form.

Keywords: Flupirtine , Thiocolchicoside ,RP-HPLC,, phenomenex luna, Validation, Stability indicating.

INTRODUCTION

Thiocolchicoside [1] (TCC) is a muscle relaxant with anti inflammatory and analgesic effects. It acts as a competitive $GABA_A$ receptor antagonist and also glycine receptor antagonist with similar potency and nicotinic acetylcholine receptors to a much lesser extent. It has powerful convulsant activity and should not be used in seizure-prone individuals. Chemically it is known as (s)-N-[3-D-glucopyranoxyloxy)-5, 6, 7, 9 tetrahydro-1,2-dimethoxy-10-(methylthio- 9- oxobenzo(a) heptalen- 7yl] acetamido. Flupirtine[2] (FLU) is an aminopyridine that functions as a centrally acting non- opioid analgesic. It is unique among analgesics in that it is a non-opioid, non-NSAID, non-steroidal centrally acting analgesic. Its muscle relaxant properties make it popular for back pain and other orthopedic uses, but it is also used for migraines, in oncology, postoperative care, and gynecology. It is chemically Ethyl {2-amino- 6-[(4- fluorobenzyl) amino] pyridin-3-yl} carbonate. Literature survey revealed that there are few analytical methods[3-12] reported for the determination of TCC and FLU in single or in combination with other drugs. However, no RP- HPLC method was reported for the evaluation of stability indicating method for the determination of TCC and FLU

MATERIALS AND METHODS

A shimadzu HPLC system consist of LC-10AT-vp Solvent delivery system (pump), SPDM– 10 AVP photodiode array detector, Rheodyne injector with20µL loop volume, LC Solution assisted for data collections and processing. The mobile phase consisted of 5 % of acetonitrile and 95 % of Phosphate buffer was delivered at a flow rate of 1 mL/min. Separation was achieved using a 150mm X4.6 mm (i.d.) Phenomenex luna C18 column with an average particle size of 5µ and the column was kept at an ambient temperature. The column effluent was monitored at 260 nm. The mobile phase was filtered through 0.45µ filter before using.

CHEMICALS AND REAGENTS

TCC and FLU pure drug was obtained as gift sample from Bio-leo analytical labs, Hyderabad. KETOFLAM T4 is the dosage form purchased from local pharmacy. Other chemicals all are of HPLC grade (Table 1).

Preparation of Phosphate Buffer Solution

6.8 gm of potassium di - hydrogen orthophosphate(25 mM) was dissolved in sufficient water (HPLC grade) with aid of sonicator. Then 5 ml of tri ethanol amine was added and the volume was made up to1000ml with mobile phase. Finally, pH was adjusted to 5 with potassium hydroxide.

Standard stock solution preparation

Accurately weigh and transfer 100 mg of FLU working standard and 4 mg of TCC working standard in to 100 mL volumetric flask, add 50 mL of mobile phase and sonicate to dissolve and dilute to volume with mobile phase. Transfer 1 mL of standard stock solution into 10 mL volumetric flask and to volume with mobile phase.

Sample Preparation

Twenty tablets containing 100 mg of Flupritine maleate and 4 mg of Thiocochicoside were weighed and average weight was calculated. An amount of powder equivalent to 400 mg of Flupritine maleate and 16 mg of Thiocolchicoside were transferred to 50 ml volumetric flask, added 20 ml Acetonitrile and sonicated for a few minutes. A 30 ml of portion of Acetonitrile was then added and sonicated for 15 min to ensure complete extraction. This solution was centrifuged at 4000 rpm for 10 min. Aliquots of this solution were transferred to 10 ml volumetric flasks and diluted with Potassium dihydrogen phosphate and Acetonitrile in the ratio of 65:35 v/v to obtain concentrations in the linearity range.

RESULTS

Method Validation

The developed method was validated according to the International Conference of Harmonisation (ICH) guidelines for validation of analytical procedures.

Linearity

Linearity is ability the method to produce results that is directly proportional to the concentration of the analyte in samples with given range(fig 3,4). The linearity of FLU was in the concentration range of 50-250µg/ml.for TCC 4-20 µg/ml .From the linearity studies calibration curve as plotted and concentrations were subjected the least square regression analysis to calculate regression equation. The regression coefficient found to be 0.9999 as shows good linearity for both the drugs (table 4)

Specificity

The specificity of the developed method was evaluated by studying the peak purity index values. Spectral purities of Flupritine 4.3 maleate and Thiocolchicoside 8.3 were evaluated. In addition, solution containing a mixture of the tablet excipients were prepared using the sample preparation procedure and injected on to chromatograph (Table 3)

Precision

The precision the method was determined repeatability (intra-day) and intermediate (inter-day) precision and was expressed as RSD of a series and measurements. Intraday precision was evaluated by six replicated reading at three concentrations levels within a linearity range. Inter-day precision was studied by comparing the results on 3 different days (table 5,6)

Accuracy

Accuracy the method was evaluated by standard addition method. Recovery the method as determined by spiking an amount of the pure drug (50%,100% ,150%) three different concentration levels it solution has been added the pre analyzed working standard solution the drug.

LOD & LOQ

LOD is the lowest concentration of analyte a sample that can be detected but not quantified under experimental conditions. The LOD values were determined the formulae LOD=3.3σ/s. LOQ the lowest concentration of analyte a sample can be determined with acceptable precision and accuracy under experimental conditions. It is parameter the quantitative determination of compounds in the mixtures. The LOQ values were determined by the formulae LOD=10σ/s

Robustness

Evaluated by studying the influence small deliberate variations of the analytical parameters on the elution, peak are or peak shape. The method should be robust enough with respect to all critical parameters as to allow routine laboratory use (Table 7,8)

Acid Hydrolysis

Sample quantity equivalent to 4 mg of TCC and 100 mg of FLU in to 200 ml was transferred in to RB flask. 100 ml of freshly prepared 0.1 Hydrochloric acid was added and refluxed for 30mins at 60^{0}c Leave it for 12 Hrs. After 12 hrs filter the solution through filter paper and neutralize the solution with suitable Base. Dilute 1 ml of filtrate to 10 ml with mobile phase).

Base Hydrolysis

sample quantity equivalent to 4 mg of TCC and 100 mg of FLU into 200 ml was transferred into a RB flask add 100 ml of freshly prepared 0.1 N sodium hydroxide was added and refluxed for 30min at 60^{0}c. Leave it for 12 h . After 12 h filter the solution through filter paper and neutralize the solution with suitable acid. Dilute 1 ml of filtrate to 10 ml with mobile phase.

Oxidation (Peroxide)

Transfer sample quantitatively equivalent to 4 mg of TCC and 100 mg of FLU in to 200 ml RB flask add 100 ml of freshly prepared 10% Hydrogen peroxide solution. The solutions were kept for 30 min at 60^{0}c. Leave it for 10 Hrs. After 10 hrs filter the solution through filter paper. Dilute 1 ml of filtrate to 10 ml with mobile phase.

UV Exposure

Sample quantity equivalent to 4 mg of thiocolchicoside and 100 mg of Flupirtine was transferred on to clean and dry petri dish. Place the petri dish in UV chamber by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. After exposure transfer the contents in to 100 ml volumetric flask and add 25 ml of mobile phase and sonicate to dissolve. Then dilute to volume with mobile phase. Further filter the solution through filter paper. Dilute 1 ml of filtrate to 10 ml with mobile phase.

DISCUSSION

Several trials has made until getting good peak resolution, acceptable plate count tailing factor. Method as optimized the retention times of TCC and FLU maleate was reported as 8.3 and 4.3 min

REFERENCES

1. http://en.wikipedia.org/wiki/Thiocolchicosi de
2. http://en.wikipedia.org/wiki/Flupirtine
3. Ramakrishna M, Venkata S Rao Somisetty, Rambabu P, Sudhakarbabu AMS and Ramana MV. Development of new analytical methods for the estimation of flupirtine maleate in bulk and in formulations. World Journal of Pharmaceutical Research. 2014;3(4):1902.
4. Mohan Gandhi B, Lakshmana Rao A and Venkateswara Rao J. A novel stability- indicating RP-HPLC method for the estimation of flupirtine in pharmaceutical dosage forms. IJPCBS. 2014;4(2):208- 218.
5. Haritha P, Sreenivasa Rao B and Sunandamma Y. Method development and validation for simultaneous determination of flupirtine maleate and paracetamol by RP – HPLC technique. International journal of pharmaceutical sciences and research. 2014;5(2):463- 472.
6. Lalitha KV, Golla Murali Mohan J, Ravindra Reddy, Vinod Kumar K and Aliekya A. RP-HPLC Method Development and Validation for the Simultaneous Estimation of Paracetamol and Flupiritine Maleate in Pharmaceutical Dosage. Journal of Scientific and Innovative Research. 2013;2(3):634-641.
7. Sanjeev Kumar, Amith Joshi, Rahul Singh Thakur, Anupam K Pathak and Kamal Shah. Simultaneous estimation of etoricoxib and thiocolchicoside by RP- HPLC method in combined dosage forms. Acta poloniae pharmaceutica. 2011;68(6):839-43.
8. Rajan V. Rele and Swapnil A. Sawant. Simultaneous Determination of Aceclofenac and Thiocolchicoside in Formulation by Reversed Phase High Performance Liquid Chromatography. American journal of pharmaceutical research. 2012;2(4):332.
9. Umarkar AR, Rewatkar NS, Charde MS, Charde RM and Kasture AV. RP-HPLC method development and validation for estimation of thiocolchicoside and diclofenac potassium in bulk and capsule dosage forms. Journal of pharmacy research. 2011;4(5):1307.

10. Satyanarayana MV, Satyadev TNVSS, Ganji Ramanaiah and Anuradha V. Method development and validation of stability indicating RP-HPLC method for simultaneous estimation of thiocolchicoside and diclofenac in bulk and its pharmaceutical formulations. Indo American Journal of Pharmaceutical Research. 2014;4(3).
11. Bhavin P Marolia , Divyesh J Vanparia, Bhavik H Satani, Pintu B Prajapati, Shailesh A Shah and Dinesh R Shah. Application of RP-HPLC method for simultaneous estimation of Flupirtine and Thiocolchicoside maleate. American journal of pharmaceutical research. 2012;2(3):244.
12. Satheesh BS, Ree Ganesh KK, Saravanan D, Gundu Ramakant K, Sivaraman and Sivananthan. Quantification of Flupirtine and its related compounds in pharmaceutical dosage forms by UPLC. Journal of Pharmacy Research. 2012;5(5):2694.

Table 1: Details of marketed Formulation

Brand name	Formulation	Available strength		Address of manufacturer
KETOFLAM T4	Tablet	Thiocolchicoside Flupirtine maleate	4mg 100mg	Lupin laboratories Ltd.
KETOFLAM T4	Tablet	Thiocochicoside Flupirtine maleate	8mg 100mg	Lupin laboratories ltd.

Table 2: Optimized chromatogram conditions

Instrument	High Performance Liquid Chromatography Waters HPLC 2 2695
Stationary phase	Phenomenex luna C_{18} (25cm 4.6mm),
Flow rate	5µ 1ml/min
Operating temperature	Ambient
Selected wave length	UV 260nm
Mobile phase ratio	Phosphate buffer
Injection volume	Acetonitrile (63:35v/v)
Run time	20µl 4.3ml/min

Table 3: Specificity Data

Name of solution	Retention time (min)
Blank	No peaks
Thiocolchicoside	8.3
Flupirtine	4.3

Table 4: Linearity study

Peak area	Concentration (µg/ml)	Peak area
94295	100	328490
187360	200	651770
280856	300	973266
366504	400	1259013
459825	500	1574373
556396	600	1931704
	Correlation coefficient	0.9995

Table 5: Precision Data (System Precision)

	Thiocolchicoside		Flupirtine maleate	
	RT	Area	RT	Area
	8.3	3798	4.3	25990
	8.2	3824	4.29	25865
	8.1	3782	4.28	26123
	8.0	3742	4.25	25925
	7.8	3841	4.23	25867
	7.6	3722	4.19	26121
Avg	8	3.784	4.256	25.981
Std dev	0.192	38.455	0.029	107.457
RSD	0.084	1.22	0.057	0.453

Table 6: Precision Data (Method Precision)

	Thiocolchicoside		Flupirtine maleate	
	RT	Area	RT	Area
	8.3	3842	4.3	26115
	8.2	3781	4.29	25921
	8.1	3832	4.28	25712
	8.0	3845	4.25	25852
	7.8	3795	4.23	26724
	7.6	3821	4.19	26121
Avg	8	3.819	4.2556	26.074
Std dev	0.192	29.129	0.02915	396.072
RSD	0.103	0.682	0.035	1.36

Table 7: Robustness Study for Thiocolchicoside And Flupirtine Maleate

Drug	Parameters	Variation	Retention time (min)
THIOCOLCHICOSIDE	Flow rate	0.8ml	8.1
		1.2ml	8.2
	Mobile phase	70:30 Organic Phase	8.13
		80:20 Organic Phase	Peak merged
FLUPIRTINE MALEATE	Flow rate	0.8ml	4.3
		1.2ml	4.28
	Mobile phase	70:30 Organic Phase	4.19
		80:20 Organic Phase	Peak merged

Table 9: Summary of validation parameters

Parameters	Thiocolchicoside	Flupirtine maleate
Linearity	50-250 µg/m	4-20 µg/ml
Precision (% RSD)	0.084 (Rt)	0.057(Rt)
Accuracy	0.260(Area)	0.103(Area)
	99.89%	100.03%
LOD & LOQ	1.252,1.634	0.853,2.513
Assay	99.80%	100.72%.

Table 10: Summary of Forced degradation data

Parameter	% RSD	
	Flupritine maleate	Thiocolchicoside
0.1 HCl	0.078	0.62
0.1 N NaOH	1.48	1.9
3% H_2O_2	0.44	1.34

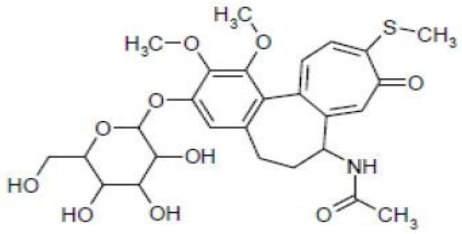

Fig. 1: Structure of Thiocolchicoside

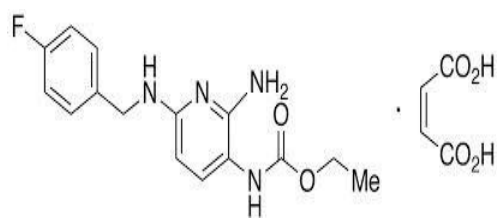

Fig. 2: Structure of flupirtine maleate

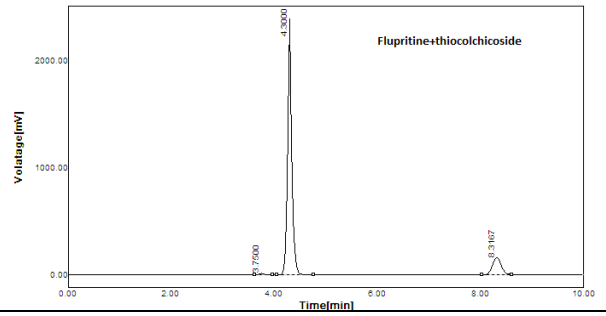

Fig. 3: Standard chromatogram of Flupritine maleate (50µg) and Thiocolchicoside(4µg)

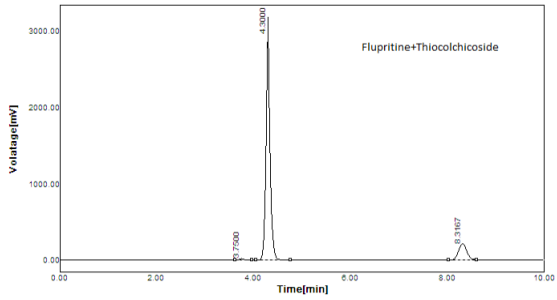

Fig.4: Standard (fig 2) chromatogram of Flupritine maleate (100µg) and Thiocolchicoside (8 µg)

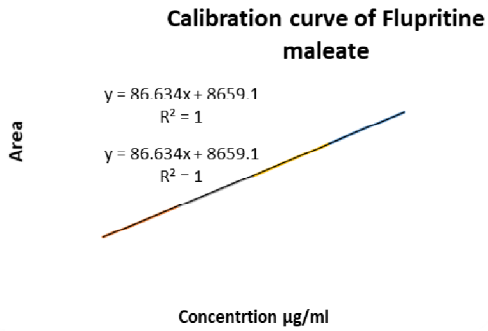

Fig.5: Linearity Graph for Flupritine maleate

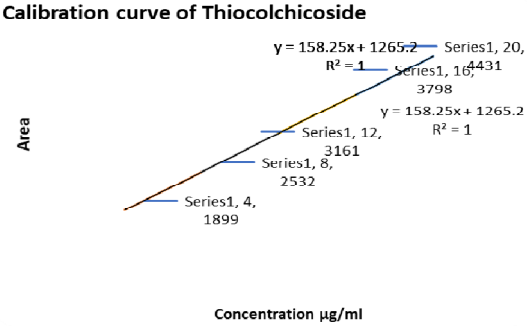

Fig.6: Linearity range for Thiocolchiside

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REVIEW ARTICLE

Nanotechnology-Based Targeted Drug Delivery Systems for Brain Tumors

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ABSTRACT

Brain tumors develop from unregulated proliferation within the brain of neural tissue cells or supporting cells of glial tissue. Brain tumor diagnosis and treatment is an incredibly difficult process. In addition, its severity is compounded by the lack of early stage signs and subsequently delays in diagnosis and therapy. Although the chemotherapeutic approach is the most popular therapeutic approach at present, it is still related to many precincts. The key obstacle to administering most chemotherapeutic agents is the blood-brain barrier (BBB), which leads to inadequate aggregation at the tumor site and prevents adequate destruction of malignant cells. Targeted delivery of nanoparticle-mediated drugs may significantly decrease dosage and optimize their release properties, increase specificity and bioavailability, boost shelf life, and decrease toxicity. A detailed overview of preclinical and clinical research of nanodrugs in brain tumor therapy is presented in this analysis, based both on a literature study and on the authors' own experimental work.

Keywords: Brain, Blood Brain Barrier, Brain Tumour, Drug Targeting

INTRODUCTION

Brain

The brain is one of the body's biggest and most muddled organs. It is comprised of more than 100 billion nerves that speak with one another through trillions of synapses. The brain is comprised of various particular areas that team up. The cortex is the brain's furthest layer. The cortex is where wondering and voluntary actions start. Among the spinal chord and the the rest of the brain is the brain stem. Respiration and sleep are basic functions which might be controlled here [1].The basal ganglia are a collection of systems inside the brain's middle. The basal ganglia sends and receives messages from many components of the brain. The cerebellum is positioned on the brain's base and back. The cerebellum is in rate of balance and coordination. The brain is divided into lobes as nicely. Hassle solving, judgement, and motor characteristic are all governed by way of the frontal lobes. Sensation,

penmanship, and body posture are all managed by way of the parietal lobes. Memory and listening to are aided with the aid of the temporal lobes. The visual processing device of the brain is housed inside the occipital lobes [2].

Blood Brain Barrier (BBB)

The BBB is a distinctly selective semipermeable border of endothelial cells that inhibits non-selective passage of solutes inside the circulating blood into the extracellular fluid of the important apprehensive machine, where neurons live. Endothelial cells of the capillary wall, astrocyte end-feet ensheathing the capillary, and pericytes imbedded within the capillary basement membrane create the blood–brain barrier. This gadget offers for passive diffusion of certain molecules in addition to selective and active shipping of nutrients, ions, natural anions, and macromolecules like glucose, water, and amino acids that are crucial for brain characteristic [3]. The blood–brain barrier prevents infections, solutes inside the blood, and large or hydrophilic molecules from coming into the cerebrospinal fluid, even as allowing hydrophobic molecules (O2, CO2, hormones) and small non-polar molecules to skip through. Barrier cells use unique delivery proteins to actively switch metabolic products such as glucose through the barrier. The barrier also prevents peripheral immunological components together with signalling molecules, antibodies, and immune cells from entering the CNS, protecting the brain from damage because of immunological events. The circumventricular organs and choroid plexus, which play a role in sensory and secretory integration within brain neuronal circuits, have extraordinarily permeable capillaries [4].

Blood-Cerebrospinal Fluid Barrier

The blood-cerebrospinal fluid barrier (BCSFB), that is positioned on the choroid plexus and separates the blood from the cerebrospinal fluid (CSF), which runs within the subarachnoid area surrounding the brain, is the second one barrier. The capillaries in the choroid plexus, in contrast to those that make up the BBB, enable unrestricted passage of molecules through intracellular gaps and fenestrations [5]. On the CSF (apical) facet of the epithelial cells inside the choroid plexus that make up the BCSFB, there are complex tight junctions. The epithelial cells of the choroid plexus have barely more permeable tight junctions than the endothelial cells of the BBB [6].

Brain Tumor and Cancer

A brain tumor is an abnormal mobile increase or mass in the brain. There are many awesome sorts of brain tumors. Some brain tumors are benign (noncancerous), even as others are cancerous (malignant). Brain tumors can begin inside the brain (primary brain tumors) or unfold from other parts of the frame to the brain (secondary brain tumors) (secondary, or metastatic, brain tumors). How quickly a brain tumour grows varies extensively [7]. The growth rate and location of a brain tumor dictate how it's going to have an effect on the nervous system's characteristic. Primarily, brain tumors increase in the brain or nearby tissues, along with the meninges (brain covering membranes), cranial nerves, pituitary gland, or pineal gland. Ordinary cells accumulate flaws (mutations) of their DNA, ensuing in primary brain tumours. These mutations cause cells to divide and expand at a quicker rate, letting them live whilst wholesome cells could die, as a end result, a tumour is formed from a mass of aberrant cells. Primary brain tumours are a long way less regularly occurring in adults than secondary brain tumours, which can be cancers that start some place else and flow to the brain. Primary brain tumours come in a selection of styles and sizes. The sort of cells concerned gives each of them its call [8].

Here are several examples

Gliomas: Astrocytomas, ependymomas, glioblastomas, oligoastrocytomas, and oligodendrogliomas are cancers that begin in the brain or spinal chord [9].

Meningiomas: Those are benign tumours that broaden inside the brain and spinal chord. A meningioma is a kind of tumour that develops inside the membranes that surround your brain and spinal cord (meninges). The majority of meningiomas are benign [10].

Acoustic neuromas (schwannomas): These are benign tumours that shape on the nerves that manipulate balance and listening to within the inner ear and result in the brain. Those are benign tumours that shape at the nerves that manipulate stability and listening to inside the internal ear and result in the brain [11].

Pituitary Adenomas: Those are benign tumors of pituitary gland positioned at the bottom of the brain, is home to most of the people of these benign tumours. Those tumours can disrupt pituitary hormones, that may have some distance-achieving results at some stage in the frame [12].

Medulloblastomas: These are tumours that expand within the brain. The maximum common malignant brain tumours in youngsters are these. A medulloblastoma is a sort of brain tumour that begins inside the backside returned of the brain and spreads via the spinal fluid. Adults are less in all likelihood to get these tumours, despite the fact that they do manifest [13].

Germ mobile tumours: Germ cellular tumours can increase in the testicles or ovaries all through youth. But, germ mobile cancers can on occasion unfold to different areas of the body, including the brain [14].

Craniopharyngiomas: Those are noncancerous tumours that begin close to the pituitary gland within the brain, which secretes hormones that regulate several body functions. The pituitary gland and other tissues across the brain may be affected while the craniopharyngioma expands [15].

Blood-Tumor Barrier

When the goal is a CNS tumour, drug transport will become significantly greater hard. The BBB's presence inside the microvasculature of CNS malignancies has healing implications. A variable distribution of microvasculature throughout the tumour interstitial compromises medication transport to neoplastic cells, resulting in spatially choppy drug transport [16]. Furthermore, whilst a tumour grows large, the vascular floor vicinity shrinks, lowering the amount of blood-borne chemicals exchanged trans-vascularly. On the identical time, intra-capillary distance will increase, resulting in a better diffusional requirement for drug transport to neoplastic cells, and increased hydrostatic pressure inside the ordinary brain parenchyma adjacent to the tumour due to excessive interstitial tumour strain and associated peri-tumoral edoema [17]. As a result, the cerebral microvasculature in these tumor-adjoining everyday brain areas may be even less drug-permeable than ordinary brain endothelium, resulting in extraordinarily low greater-tumoral interstitial drug concentrations. BBB can be disrupted by using brain tumours, despite the fact that these disruptions are localised and nonhomogeneous [18]. Sooner or later, a number of difficult obstacles, including as the BBB, the BCB, and the BTB, often save you drugs from accomplishing the CNS via the circulatory machine. Furthermore, new research has revealed the possibility of achieving the brain via the nasal route. Certainly, it has been validated that through this channel, drug shipping across the olfactory vicinity of the nasal hollow space happens, directly reaching brain tissue or CSF [19]. It is based on the olfactory bulb, that's the connection among the nostril and the brain. The olfactory epithelium is located between the nasal septum and the lateral walls of each of the two nasal cavities, without delay underneath the cribriform plate of the ethmoid bone that divides the nasal cavity from the cranial hollow space.[20]

Nanotherapeutics Platforms for Brain Cancer Applications

Gold Nanoparticles (AuNPs)

AuNPs crafted from gold cores are a primary system with distinguishing characteristics for theranostic structures. They are biocompatible, and they may be usually geared up to take. Chemically, hydrogen tetrachloroaurate is transformed into spheres, cubes, rods, cages, and chords, which are typically used as spheres, cubes, rods, cages, and wire. The ease with which researchers can tailor AuNPs to numerous sizes, shapes, and functionalities permits them to delve deeper into the capacity packages of AuNPs as nanotheransotics, mainly for cancer prognosis and remedy.[21] AuNPs, like different inorganic nanoparticles, showed a cytotoxic effect brought about by means of oxidative pressure. AuNPs, like other inorganic nanoparticles, have a deadly impact while exposed to oxidative stress. The spheroidal AuNPs (size 10 nm) have a specific UV absorption at 520 nm, and size variations correspond to crimson or blue shifts. The absorption movements to the close to-infrared radiation (NIR) area (690nm – 900nm) when gold nanorods are used. [22] AuNPs can be hired as a spread of theranostic marketers in medical programs due to their intrinsic optical residences. Diagnostic homes, surface plasmon absorption,

monodispersity, huge surface to extent ratio, low toxicity, tunable core length, capability to connect with biomolecules through Au–Sulphur bonds, simplicity of manufacture, and mild-scattering abilities are all superior aspects of AuNPs. Photoacoustics, CT, and surface-improved Raman spectroscopy are only some of the imaging-related applications that have been investigated (SERS). [23]

As complicated theranostic systems, AuNPs may be manufactured with therapeutic compounds and focused on moieties that particularly realise the receptor at the goal place for lively focused on. An electrostatic touch or covalent conjugation is used to load a medicinal molecule. Some Scientists as an instance, created multi-useful gold-based totally nanoshells with magnetic and optical talents, which had been then coupled with a targeting moiety and examined to be used in neck and head most cancers. [24] Whilst as compared to radiation remedy alone, a singular concept inclusive of the utilisation of AuNPs for radiation therapy has notably stepped forward long-term survival. It confirmed promise in phrases of improving glioma treatment. Some Scientists synthesised AuNPs functionalized with PEG, biotin, PTX to the surface, and rhodamine B connected beta-cyclodextrin (b-CD) (AuNPs-50) as a theranostic device for cancer remedy with no harmful impact on ordinary cells in every other examine. [25] AuNPs have verified the capacity to administer fluorescent standards, reveal medicinal drug distribution, and purpose laser-induced most cancers cell destruction. In this observe, varieties of AuNPs, AuNPs-4 and AuNPs-5, had been tested for his or her capability to have interaction with tumour cells. Those have been additionally tested in opposition to easy NIH3T3 cells, and it become determined that the AuNPs-5 have been extra effectively interacting with the tumour cells, as proven by using fluorescence and cell viability tests . [26]Moreover, AuNPs can be used as a promising candidate for intraoperative tumour margin identity, which improves brain tumour surgical procedure . Some Scientists synthesised Tf peptide centered AuNPs (Tf-AuNPs) of a photodynamic drug, Pc 4, and as compared them to non-focused AuNPs for anti-cancer drug delivery to brain tumour mobile lines in a single research [27]. Moreover, in-vitro investigations on cellular lines found out a massive development in focused formulation uptake research in comparison to non-focused debris. In a latest observe, pH-brought about launch of a concentrated on peptide RGD and octarginine with a concentrated on peptide RGD and octarginine for glioma unique targeting turned into achieved using matrix metalloproteinase-2 touchy gold-gelatin nanoparticles. [28] An in-vivo examine verified that glioma focused on the use of gold nanotheransostics became a hit with co-localization within neovessels. Gold-magnetic theranostic micelles covered with polyethylene glycol-polycaprolactone (PEG-PCL) polymer have been created as a multimodal method and demonstrated radio sensitising efficacy for brain most cancers therapy. It was additionally proposed as a new assessment agent for MRI and CT examinations.[29]

Magnetic Nanoparticles (MNPs)

These days, MNPs have showed promise as nanocarriers for centered medication delivery at most cancers websites, with the introduced gain of MRI traceability. Several studies have formerly confirmed that these nanoparticles can be retained in cancer locations while mixed with an externally generated magnetic discipline.[30] The magnetic reaction of the iron oxide middle permits magnetic focused distribution. Furthermore, it has been validated that traceable quantities of magnetic nanoparticles can attain the tumour website of 9L-glioma-bearing animals after intravenous remedy. Iron oxide nanoparticles (IONPs) commonly have a magnetic center (e.g., magnetite/iron oxide) and an outer polymeric shell (starch, dextran and many others). [31] Due to their superparamagnetic outcomes, low price, and suitable biocompatibility, they gift a capacity method for software in theranostics, allowing researchers to rent in several organic applications inclusive of contrasting probes in MRI. The IONPs are utilized in magnetite or hematite shape. It is straightforward and handy to synthesise IONPs using thermal decomposition and co-precipitation strategies. Moreover, surface modification of MNPs with various inorganic molecules, polymeric and non-polymeric stabilisers, and ligands facilitates the usage of IONPs-loaded sellers in theranostic packages.[32] Polyvinyl pyrrolidone (PVP), polyaniline dendrimers, and dextran are among of the ligands used for the aforementioned dreams. In keeping with reviews, IONPs are biocompatible materials for the reason that they decay in the biological system and are metabolised into the serum iron pool as haemoglobin. Dextran and its derivatives have received the greatest interest.[33] In fact, a couple of dextran–MNPs formulations are currently or have these days been authorised to be used as MRI evaluation agents in clinical trials. Apart from

theranostic homes, MNPs have a extremely good transverse relaxation time (T2) compared to other MRI assessment agents, hyperthermia, and immunotherapeutic cost for autoimmune ailments. [34] Some Scientists investigated the potential of magnetic nanoparticles for each magnetically better accumulation in brain cancer and non-invasive MRI screening in a have a look at. They concluded that magnetic targeting might appreciably boom the accumulation of magnetic nanoparticles in gliosarcomas, which become correctly assessed by way of MRI.[35] As a end result, these nanosystems validated to be an effective conduit for focused drug shipping. latest *in-vivo* research of MNPs with stimulus responsive characteristic, BBB targeted administration, and reversible BBB beginning with warmth also cautioned that these nanotheranostics might be utilized in scientific settings.[36]

Quantum Dots (QDs)

In 1983, Brus and his colleagues at Bell Laboratories created the primary colloidal QDs. QDs are inorganic semiconductor nanocrystals with a nanoscale (10 nm) that have emerged as flexible instruments for molecular diagnostics and nanotherapeutics. QDs have lately been pronounced as attractive diagnostic dealers for theranostic purposes, outperforming traditional organic fluorophores. [37] It's been proven that the emission wavelength of QDs can be changed from 450 to 1800 nm through modifying their form, length, and composition. QDs are binary compounds made from clusters of 10 to a 1000 atoms, along with cadmium selenide (CdSe). The maximum extensively used nanomaterials for diagnostic functions are CdSe/Zinc sulfide-primarily based QDs. [38] They've a CdSe middle this is blanketed with layers of ZnS. Further, conjugating concentrated on probes at the floor of QDs is an vital a part of centered theranostic transport. Concentrated on ligands which include peptides, antibodies, nucleic acids, aptamers, folate, and other small molecule ligands also can be attached to the floor of QDs through non-covalent and/or covalent interactions so that it will acquire affinity and targeted delivery to most cancers tissue.[39] The surface of QDs may be functionalized with moieties which includes -COOH, NH2, and SH after which conjugated with focused moieties the usage of maleimide, carbodiimide, and succinimide conjugation chemistries. Any other key approach for conjugating centered ligands at the floor of QDs is a vidin-biotin pass-linking. Many researchers have currently tried to construct theranostic nano modules for most cancers theranostics by means of integrating the increased fluorescence talents of QDs with therapeutic skills right into a single nanosystem [40]. It's been confirmed experimentally that QDs can be employed for actual-time fluorescence imaging both *in vitro* and *in vivo*. QDs may be conjugated with most cancers mobile particular ligands which include a prostate-specific antigen, HER2, folic acid, proteins, CD44, antibodies, immunoglobulins, and so on. Furthermore, investigations have shown that QDs can be integrated into paramagnetic liposomal formulations containing RGD peptides and used to perceive tumour angiogenesis the usage of MRI.[41] Presently, using quantum dots in theranostic structures has restricted medical promise because of their gradual metabolization and elimination, which can be dangerous to human systems. Surface-changed, biocompatible, and excretable QDs are especially anticipated inside the destiny [42].

Carbon Nanotubes (CNTs)

The cylindrical shape of CNTs is due to the number of layers of graphene sheets used. CNTs are carbon allotropes with sluggish biodegradability and terrible biocompatibility. They function specific electric and mechanical residences that lead them to appropriate for theranostic applications. They may be classed as fullerene, carbon nanotubes (CNTs), graphene, and carbon dots (i.e., size lesser than 10 nm)[43]. CNTs can improve most cancers chemotherapy, making them an notable candidate for healing programs. While exposed to NIR irradiation, carbon nanotubes produce lethal heat. Once in the cells, they'll have interaction with proteins and DNA, influencing mobile signalling or the mechanism of other remedies.[44] CNTs' intrinsic NIR mild absorption belongings has been hired to *in-vitro* demolish cancer cells, even as their NIR photoluminescence assets has been exploited for *in-vitro* cell imaging and probing. Robinson and co-workers validated the efficacy of intravenous shipping of single-walled carbon nanotubes (SWCNTs) as photoluminescent probes for *in-vivo* tumour imaging.[45] The work validated large benefits of utilizing the intrinsic residences of SWCNTs for theranostic programs. CNTs can enhance the remedy of brain tumours, making them extra beneficial in scientific practice. Ren et al., as an instance, developed a dual-centered PEG-based totally oxidized MWCNTs (O-MWNTs) conjugated with angiopep-2 for the treatment

of brain glioma. The impact of doxorubicin (DOX) loaded O-MWNTs (DOX-OMWNTs-PEG-ANG) on glioma treatment become investigated for cytotoxicity.[46] Certainly, gold-coated, surface-modified CNTs have been recently produced as an optical nanotheranostic probe that established the biological sample's focused Raman imaging potential using an NIR laser as an excitation supply. CNTs' healing usefulness is confined, however, due to their slower biodegradation rate, which may additionally bring about toxicity for the duration of *in-vivo* nanotheranostic programs. Furthermore, the era of unfastened radicals by means of CNTs can bring about lipid peroxidation, that could reason cellular damage and irritation in vital organs.[47]

Mesoporous Silica Nanoparticles (MSNPs)

MSNPs are another developing DDS that is being explored appreciably due to its customizable form and length, as well as their excessive floor location and void extent, which permit for significant drug loading. MSNPs are meant for both diagnostic (fluorescence imaging and/or MRI) and healing purposes (drug transport or PDT).[48] Many different medicinal drugs, including PTX, camptothecin, DOX, methotrexate, colchicine, chlorambucil, cysteine, and telmisartan, have been successfully loaded in MSNPs or covalently connected to MSNPs. MSNP-based anti-cancer medicine transport causes cancer cells to die appropriately. MSNPs, like carbon nanomaterials, have a extraordinarily organised hexagonal structure with high internal floor regions starting from 500 to 1200 m2/g due to periodic preparations of mesopores (width starting from 2 to 20 nm) encased in a silica framework. [49] Certainly, MSNPs are uniquely tailored to combine the desired capabilities of a perfect theranostic device in a single entity, with discrete sections for the healing moiety, contrasting agent, and biomolecular binding. Moreover, MSNPs is recognized as a secured material via the FDA, and an MSNPs is now permitted to be used in a scientific research.[50] Reviews on the usage of nanoparticles as opposed to small molecules for integrating into silica matrices, as well as the application of such era to encapsulate IONPs, AuNPs, and QDs, are properly documented. Moreover, many purposeful additives can be integrated into a single silica particle at the equal time. Scientists, for instance, have employed silica to incorporate each IONPs and QDs, resulting in a hybrid with both magnetic and optical abilities. [51] MSNPs are regarded biodegradable and biocompatible materials for nanotheranostic packages since the organic system might also soak up the dissolved silica of MSNPs, digest it, and excrete it as silicic acid or oligomeric silica species via the urine. They hydrolyzed at decrease quantities in physiological settings. Moreover, biomolecular focused on targeting such as peptides and proteins which are conjugated to the surface of MSNPs to deal with most cancers.[52] Trans-activating transcriptional activator (TAT) peptide-conjugated MSNPs, as an example, had been used as a nucleus-targeted DDS and proven large development in anticancer interest with the aid of nuclear internalisation.[53] The surface of MSNPs became conjugated with Tf, resulting in improved identification of brain tumour cells. MSNPs are used as evaluation parameters in ultrasound and MRI due to their rugged nature, high drug loading performance, diverse functionalization, and obvious biodegradation within the body in a well timed manner. They're also used for particular targeting with low toxicity and display superb results for brain cancer detection.[54] Huang et al. defined a mesenchymal stem cellular (MSC) centered theranostic platform (MSNPs) for orthotopic glioblastoma prognosis and remedy. The nanotheranostics were systemically injected into mice and verified excessive selectivity for the glioma location, as well as *in-vivo* imaging through NIR fluorescence, MRI, and PET[55].

Upconversion Nanoparticles (UCNPs)

UCNPs have the capacity to soak up low-strength photons while emitting excessive-strength photons. This up conversion phenomenon occurs in transition metals, actinides, however most significantly in rare earth (RE) metals, which broadly speaking include lanthanide (Ln) elegance elements like yttrium and scandium.[56] UCNPs use a nonlinear optical mechanism to generate high power visible radiation from low energy NIR radiation. The materials are inspired by soaking up low electricity radiation at longer wavelengths after which emitting better strength visible radiation through multiphoton absorption.[57] Because of this feature, they're greater popular systems for nanotheranostic programs. Due to the fact organic tissues do not take in NIR radiation from UCNPs, their stimulation causes no photodamage. Importantly, the narrow and sharp emission spectrum of UCNPs improves the performance and sensitivity of upconversion nanotheranostics for healing utilization significantly.[58]

Lanthanide-based totally UCNPs, specially, had been described as incredibly safer substances for nanotheranostic purposes, as they're unexpectedly removed through hepatobiliary and renal excretion routes. Several Research have suggested co-precipitation, thermal decomposition, hydrothermal/solvothermal, polyol, and ionic liquid-primarily based UCNP synthetic techniques.[59]The UCNPs synthesized using the techniques defined above are water insoluble because of their hydrophobicity, however they may be water dispersible to some extent in a few conditions. Moreover, surface modification is required to overcome these difficulties, that's executed with the aid of converting the surface with an inorganic shell layer and a organic capping ligand.[60] UCNPs have piqued the interest of a extensive variety of researchers in recent years. UCNPs, specifically, have grown in reputation in medical-related packages. The primary benefits of UCNPs are: i) exceptional signal-to-noise ratio and better sensitivity because of the lack of autofluorescence, ii) deeper NIR light penetration into organic tissue causes much less photo damage, and iii) easy and cheaper stimulation with low power NIR laser.[61] UCNPs also have slender emission peaks, sturdy chemical and bodily balance, giant Stokes shifts, and minimal toxicity. As a result, UCNPs provide large alternatives to traditional fluorescent probes in clinical packages. Energy transfer upconversion (ETU), photon avalanche (PA), and excited nation absorption(ESA) are the three primary mechanisms with the aid of which up-conversion luminescence processes arise.[62] Ni et al., for instance, evolved dual-focused on nanoprobes (ANG/PEG-UCNPs) able to crossing the BBB for brain most cancers remedy. In assessment to the medical product, the nanoprobes focused to glioblastoma confirmed improved imaging and focused on results, along with MRI contrast gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) and fluorescent dye five-aminolevulinic acid (five-ALA).[63] In precis, mobile line and experimental animal experiments with dual targeting nanoprobes generated through covalent attachment of Angiopep-2 (ANG) with PEG-lined UCNPs shown that those nanoprobes may want to penetrate the BBB through receptor-mediated transcytosis and acquire glioblastoma mobile goal. They've improved the accuracy of surgical resection, preoperative analysis, and intra-operative positioning of intracranial glioblastoma, which previously had the chance of incomplete excision due to the tumor's invasive nature [64].

Polymeric Nanoparticles (PNPs)

PNPs had been determined to have a selection of blessings in drug delivery to the CNS because of their capability to entrap pharmaceuticals, stopping them from metabolism and excretion, in addition to in the transfer of anti- cancer medicines via the BBB without affecting the barrier characteristics .[65] PNPs are strong nano-sized colloidal particles wherein an anti-cancer medicine is dissolved, entrapped, encapsulated, or adsorbed onto a polymer matrix. They were shown to improve the healing efficacy of a huge range of water soluble/insoluble medicinal compounds, together with anticancer drugs,[66] Through improving bioavailability, solubility, and retention time. Chitosan, gelatin, albumin, and sodium alginate are examples of herbal polymers used for nanoparticle formation, whilst artificial polymers encompass PLA, PLGA, poly-glutamic acid, polyglycolide, and poly anhydride. The polymer's physicochemical properties, inclusive of crystallinity, molecular weight, hydrophobicity, and poly dispersity index, have been observed to regulate dissolution and drug delivery kinetics. They can be made using an expansion of strategies which includes nanoprecipitation, solvent evaporation, emulsification/solvent diffusion, and salting out. [67] Normally, biodegradable, biocompatible, clinically feasible, and much less poisonous polymers, along with PLA, PLGA, and their copolymers, are preferred within the fabrication of nanotheranostics, as they're easily eliminated after normal metabolic pathways. [68] In a recent have a look at, much less aggregable brain-penetrating PNPs for drug delivery had been evolved and pronounced. Some other patent described polymethacrylic acid-primarily based nanoparticles with polysorbate moieties for focused brain delivery.[69] PLGA, for example, is the maximum efficiently used biodegradable polymer because its hydrolysis produces constituent monomers, lactic acid, and glycolic acid, which can be certainly gift in the frame and are quickly metabolised and excreted. Pinocytosis and clathrin-mediated endocytosis were used to move those PLGA nanoparticles.[70] Poloxamer 188 lined PLGA nanoparticles containing DOX have been capable of go the BBB and decrease tumour growth in a rat version in a single have a look at. Some Scientists created PTX-loaded RGD-grafted PLGA-NPs to target tumour endothelium and improve efficacy. The ligands were attached to the PEG chain of the nanoparticles' poly caprolactone-beta-poly ethylene glycol (PCL-b-PEG).[71] In keeping with the

findings of the in-vitro have a look at, RGD-attached nanoparticles have been highly internalised into Human Umbilical Vein Endothelial cells (HUVEC) by using selectively binding to αvβ3 integrin. They established that concentrated on RGD-grafted nanoparticles to tumour vessels reduced the growth of the transplantable lymphoid tumour (TLT) and increased survival times in mice given RGD-nanoparticles of PTX.[72] Lengthy-circulating nanoparticles fabricated from methoxy poly (ethylene glycol)-polylactide or poly(lactide-co-glycolide) (mPEG-PLA/PLGA) had exact safety and balance profiles and produced sustained drug. launch. Because functionalized PEG-PLA changed into readily to be had, it enabled the production of targeted nanoparticles via conjugating it with unique mobile floor ligands.[73] The usage of anti-transcytosis receptor peptidomimetic antibodies to the BBB, brain glioma focused PEGylated immune nanoparticles were created that had been able to turning in anti-cancer agents into the mind parenchyma without changing BBB permeability. Zhan et al. created c(RGDyK)-changed polyethylene glycol–polyethylenimine (PEG–PEI) nanoparticles for intracranial gene delivery in brain cancer treatment in any other have a look at. [74] RGD receptor targeted nanoparticles had affinity for U87 cells and aided in the transport of goal unique gene for intracranial glioblastoma treatment in vivo [75].

Polymeric Micelles (PMs)

Polymeric micelles (PMs) have lately piqued the interest of researchers as flexible nanosystems for most cancers remedy focused on. Kataoka's institution proposed PMs as DDS inside the early 1990s. They created block copolymer micelles with the capacity to load and deliver a ramification of anticancer therapeutics and diagnostics at unique sites.[76] PMs are presently getting used successfully in both preclinical and scientific studies. Micelles are amphiphilic round structures with a hydrophilic shell surrounding a hydrophobic middle. Micelles have benefits including thermodynamic and kinetic stability, a higher payload, and a smaller length (much less than 50 nm). Numerous researches are being performed in an effort to increase focused on micelles for brain cancer prognosis and remedy. [77] Due to their small length and ease of practice, PMs were attracting scientists for glioma focused on in latest years. Zhan et al. organized PTX-containing cyclic (Arginine-Glycine-Aspartic acid-D-Tyrosine-Lysine)-Poly ethylene glycol-block-poly lactic acid-paclitaxel micelles [c(RGDyK)-PEG- PLA-PTX] for boosting anti-glioblastoma effect in one examine. [78] Micelles expanded cytotoxic efficiency in glioblastoma cells with the aid of 2.5-fold, in keeping with an in-vitro take a look at. c(RGDyK)-PEG-PLA micelles had been dispensed into the intracranial tumour tissue in the U87MG glioblastoma model, efficaciously inhibiting tumour increase some of the studied PTX formulations. As a result of those findings, c(RGDyK)-PEG-PLA micelles can be an effective nanotheranostic system for the treatment of integrin αvβ3 over-expressed glioblastoma. [79] Some Scientists prepared TPGS micelles of docetaxel (DTX) for brain cancer chemotherapy, which expanded cytotoxicity in brain most cancers cells threefold when as compared to a manage DTX method. When DTX micelles had been biodistributed, they lasted longer within the blood, brain, and lungs than manipulate DTX.[80] Some Scientists created a singular long-circulating, cyclic RGD-connected PMs formulation containing (1,2-diamino cyclohexane) platinum(II) (DACHPt) and the drug oxaliplatin in any other have a look at. The stay animal imaging consequences verified cRGD's tumour targeting abilities, implying that lively delivery of cRGD facilitated drug transport throughout the vascular and blood-brain tumour barrier (BBTB).[81] Finally, theranostic micelles' scientific potential for brain cancer applications changed into established by the usage of hydrophobic drug encapsulation, biocompatible polymers, flexible concentrated on aspects, a simple method of guidance, and a excessive achievement charge in preclinical studies [82].

Solid Lipid Nanoparticles (SLNs)

SLNs are every other advanced kind of nanocarrier that would be utilized in targeted drug shipping. They may be made of biocompatible lipids and are considered secure. They have got a size range of 10-1000 nm and are synthesised through dispersing lipid into water or an aqueous surfactant solution [83]. They integrate the advantages of liposomes and PNPs while final particularly stable in physiological environments. Furthermore, no toxic natural solvent is required in the instruction of SLNs, making them secure for use. They are able to incorporate each hydrophilic and hydrophobic agents, that's mainly beneficial in protein or peptide delivery. They may be regarded as a relatively adaptable platform for brain tumour imaging and therapy [84]. Numerous brain cancers

concentrated on SLNs and their *in-vitro* and *in-vivo* efficacy have been studied during the last decades. The findings of these studies had been shown to improve the efficacy of chemotherapeutic agents at the same time as reducing their aspect consequences.[85] Polysorbate-lined particles had been determined to improve CNS pharmacological consequences, while Poloxamer coatings had been located to be useless.[86]In a single take a look at, Poloxamer 188 stabilised stearic acid camptothecin-loaded SLNs were administered orally and intravenously to mice. The maximum concentration (Cmax) accelerated through 180% after i.v. administration of SLNs.[87] The Area under the curve (AUC)/dose and Mean Residence Time (MRT) of SLNs were improved by way of 10.4 and 4-fold, respectively. Jain et al. lately developed transferrin (Tf)-conjugated SLNs (Tf-SLNs) and examined them for temozolomide (TMZ) transport to the brain for GBM therapy.[88] The depth of fluorescence observed in cell uptake research was better in Tf receptor focused SLNs than in non-targeted SLNs. Martins et al. investigated the capacity of camptothecin-loaded SLNs to enter the brain parenchyma after passing through the BBB in every other examine [89]. They prepared camptothecin-loaded SLNs for brain concentrated on for this reason and confirmed that SLNs have a useful impact on brain concentrated on when as compared to the non-encapsulated drug [90].

Dendrimers

Dendrimers are a form of properly-prepared hyperbranched polymer. Buhleier et al. created cascade polymers for the primary time in 1978. They started out as small hyper-branched molecules called polypropylene imine (PPI) dendrimers, which had been synthesised inside the early 1990 with the aid of the Meijer and Mulhaupt corporations [91]. Some Scientists developed a new kind of dendrimer from a aggregate of amines and amides in 1983, the classic poly-amidoamine dendrimers, additionally called PAMAM dendrimers. Diverse styles of dendrimers were mentioned by numerous researchers in the1980's and 1990's, and the improvement of recent dendrimer designs is ongoing. The generation is defined as the wide variety of branching shells (G).[92] Dendrimers can function as drug transporters due to their properly-installed 3D shape and severa surace functional groups, and drug molecules can be loaded into the dendrimers and connected to the surface groups. [93]The abundance of reactive functional groups on their surface helps the loading of various therapeutic agents via conjugation. They often outperform different transport systems in terms of length uniformity, reduced macrophage uptake, speedy mobile entry, goal capability, and extra considerable transcytosis across biological membranes.[94] Floor changed dendrimers showcase lower cyto toxicity and better biocompatibility in *in-vivo* programs. As an example, a prodrug based on dendrimer has been advanced for PTX (p-gp substrate) to enhance drug permeation and transport throughout organic limitations. [95] Whilst as compared to PTX on my own, the lauryl-changed G3 PAMAM dendrimer-PTX conjugates showed correct balance below regular physiological situations and a 12-fold increase in permeability throughout Caco-2 cellular and porcine brain endothelial cell monolayers. Surface-modified G3 PAMAM dendrimers, in keeping with the authors, could be promising nanocarriers for lipophilic p-gp substrate pills. Sarin et al. created a G5 PAMAM dendrimer of DOX with a 7-10 nm length range for drug transport across the BBTB to GBM tumour cells.[96] Dendrimer was determined to be extensively extra effective in inhibiting the increase of RG-2 gliomas for up to 24 hours. In every other look at, Some Scientists created a polysorbate 80-lined dendrimer of DTX for the remedy of brain tumours, demonstrating an extra *in-vitro* cytotoxic potential and *in-vivo* impact on brain tumours. Some Scientists focused on gene shipping to the brain using a Tf receptor focused dendrimer as a carrier in any other study [97]. The cytotoxicity of dendrimer on bEnd.3 murine brain endothelioma cells became multiplied 1.4-fold and 2. 3-fold *in vitro*. Further Tf bearing dendrimers were proposed as a promising method for gene centered transport in brain cancer applications [98].

CONCLUSION

The BBB is a giant barrier inside the remedy of CNS sicknesses, which include brain tumours. There are currently numerous methods for transporting tablets across the BBB; however, there are limitations and challenges that must be addressed. Cutting-edge treatment does no longer offer a long-time period solution for brain tumour patients and has failed to enhance their satisfactory of existence. Nanotherapeutic approaches provide an

attractive remedy alternative for brain tumours and feature the ability to address the constraints of modern therapeutic procedures, but the lengthy-time period effects of nanotherapeutics are currently unknown, and similarly research into biodistribution, pharmacokinetics, and toxicity is required earlier than they can be utilized in scientific settings. As an end result, the important thing parameters of nanocarriers must be evaluated with the intention to increase a powerful brain-tumor focused transport gadget. In this regard, the layout of experiment method is a precious tool for screening and optimising procedure/formula parameters in nanocarrier fabrication, which has these days been carried out for a spread of therapeutic applications. Using the frame's very own cells as a drug delivery automobile is a singular approach in centered drug delivery strategies. Immune cells (monocytes, macrophages, and neutrophils) and stem cells can cross the intact BBB, probably allowing healing molecules and nanocarriers to be transported. These cellular- mediated structures represent an exciting new technique to transporting therapeutics across the BBB. No matter the interesting scientific capability, there are some boundaries, which include the encapsulated healing molecule's ability to harm circulatory cells and cells' restricted capability to successfully launch entrapped therapeutics. To broaden a price-effective and strong system, more studies are needed to optimise the surface characteristics, release profile, and biocompatibility of those novel cellular-mediated vehicles. Currently, the integration of theranostic and imaging strategies with nanotechnology (multi-functional nanocarriers) has spread out new avenues for brain tumour centered remedy. As a result, developing a multifunctional nanocarrier platform holds excellent promise and could result in interesting advances in brain tumour remedy techniques. Basic, to attain the purpose of efficient drug transport to brain tumours, a higher knowledge of the physiochemical residences of therapeutic molecules, pharmacokinetics of transport structures, and molecular mechanisms worried in BBB regulation and drug transportation is required. Collaboration among instructional and enterprise scientists is also important in developing a singular device for brain-tumor therapeutics that can increase patient survival at the same time as improving best of lifestyles.

REFERENCES

1. Kang, Y. J., Cutler, E. G., & Cho, H. (2018). Therapeutic nanoplatforms and delivery strategies for neurological disorders. *Nano Convergence*, *5*(1). https://doi.org/10.1186/s40580-018-0168-8
2. Singh, J., Garg, T., Rath, G., & Goyal, A. K. (2016). Advances in nanotechnology-based carrier systems for targeted delivery of bioactive drug molecules with special emphasis on immunotherapy in drug resistant tuberculosis – a critical review. *Drug Delivery*, *23*(5), 1676–1698. https://doi.org/10.3109/10717544.2015.1074765
3. Li, Z., Tan, S., Li, S., Shen, Q., & Wang, K. (2017). Cancer drug delivery in the nano era: An overview and perspectives (Review). *Oncology Reports*, *38*(2), 611–624. https://doi.org/10.3892/or.2017.5718
4. Wu, X., Yang, H., Yang, W., Chen, X., Gao, J., Gong, X., Wang, H., Duan, Y., Wei, D., & Chang, J. (2019). Nanoparticle-based diagnostic and therapeutic systems for brain tumors. *Journal of Materials Chemistry B*, *7*(31), 4734–4750. https://doi.org/10.1039/c9tb00860h
5. Su, H., Wang, Y., Liu, S., Wang, Y., Liu, Q., Liu, G., & Chen, Q. (2019). Emerging transporter-targeted nanoparticulate drug delivery systems. *Acta Pharmaceutica Sinica B*, *9*(1), 49–58. https://doi.org/10.1016/j.apsb.2018.10.005
6. Li, X., Tsibouklis, J., Weng, T., Zhang, B., Yin, G., Feng, G., Cui, Y., Savina, I. N., Mikhalovska, L. I., Sandeman, S. R., Howel, C. A., & Mikhalovsky, S. V. (2017). Nano carriers for drug transport across the blood–brain barrier. *Journal of Drug Targeting*, *25*(1), 17–28. https://doi.org/10.1080/1061186X.2016.1184272
7. Liu, Y., & Lu, W. (2016). Recent advances in brain development. *Journal of Chemical Neuroanatomy*, *75*, 1. https://doi.org/10.1016/j.jchemneu.2016.06.005
8. Ramos-cabrer, P. (2013). *Liposomes and nanotechnology in drug development : focus on neurological targets*. 951–960.
9. Woldu, M. A., Lenjissa, J. L., & Satessa, G. D. (2014). *Design and Evaluation of Nanoparticulate Drug Delivery Systems for Imaging and Treatment of Malignant Brain Tumor*. *14*(1).
10. Datta, N., Pal, M., Roy, U., Mitra, R., & Pradhan, A. (2014). World Journal of Pharmaceutical Research. *Infection*, *13*(3), 15. https://doi.org/10.20959/wjpr20203-16964
11. Roy Sandipan. (2012). Strategic Drug Delivery Targeted to the Brain: A Review. *Pelagia Research Library*, *3*(1), 17.

12. Zhao, M., van Straten, D., Broekman, M. L. D., Préat, V., & Schiffelers, R. M. (2020). Nanocarrier-based drug combination therapy for glioblastoma. *Theranostics*, *10*(3), 1355–1372. https://doi.org/10.7150/thno.38147
13. Shakeri, S., Ashrafizadeh, M., Zarrabi, A., Roghanian, R., Afshar, E. G., Pardakhty, A., Mohammadinejad, R., Kumar, A., & Thakur, V. K. (2020). Multifunctional polymeric nanoplatforms for brain diseases diagnosis, therapy and theranostics. *Biomedicines*, *8*(1). https://doi.org/10.3390/biomedicines8010013
14. Mohanta, B. C., Palei, N. N., Surendran, V., Dinda, S. C., Rajangam, J., Deb, J., & Sahoo, B. M. (2019). Lipid Based Nanoparticles: Current Strategies for Brain Tumor Targeting. *Current Nanomaterials*, *4*(2), 84–100. https://doi.org/10.2174/2405461504666190510121911
15. Alexander, A., Agrawal, M., Uddin, A., Siddique, S., Shehata, A. M., Shaker, M. A., Rahman, S. A. U., Abdul, M. I. M., & Shaker, M. A. (2019). Recent expansions of novel strategies towards the drug targeting into the brain. *International Journal of Nanomedicine*, *14*, 5895–5909. https://doi.org/10.2147/IJN.S210876
16. Sonali, Viswanadh, M. K., Singh, R. P., Agrawal, P., Mehata, A. K., Pawde, D. M., Narendra, Sonkar, R., & Muthu, M. S. (2018). Nanotheranostics: Emerging strategies for early diagnosis and therapy of brain cancer. *Nanotheranostics*, *2*(1), 70–86. https://doi.org/10.7150/ntno.21638
17. Patel, D. J., Mistri, P. A., & Prajapati, J. J. (2012). Treatment of cancer by using nanoparticles as a drug delivery. *International Journal of Drug Development and Research*, *4*(1), 14–27.
18. Anand, A., Sugumaran, A., & Narayanasamy, D. (2019). Brain targeted delivery of anticancer drugs: Prospective approach using solid lipid nanoparticles. *IET Nanobiotechnology*, *13*(4), 353–362. https://doi.org/10.1049/iet-nbt.2018.5322
19. Kesharwani, P., & Gupta, U. (2018). Nanotechnology-based targeted drug delivery systems for brain tumors. In *Nanotechnology-Based Targeted Drug Delivery Systems for Brain Tumors* (pp. 1–462). https://doi.org/10.1016/C2016-0-03390-9
20. Nishikant, D., Shiva, S., Rishu, T., & Vikrant, C. (2016). Nanotechnology as a targeted drug delivery system to brain. *Nanoscience and Nanotechnology*, *10*(5), 1–9.
21. Amiji, M. (2015). Nanotechnology for CNS delivery of biological therapeutics. In *The Biomedical & Life Sciences Collection*. https://hstalks.com/t/3034/nanotechnology-for-cns-delivery-of-biological-ther/
22. Polymeric Nanoparticles for Drug Delivery to the Brain. (2013). In *Polymeric Nanomedicines* (pp. 340–411). https://doi.org/10.2174/978160805484811301001 3
23. Anajwala, C. C., Jani, G. K., & S.M., V. S. (2010). Current Trends of Nanotechnology for Cancer Therapy. *International Journal of Pharmaceutical Sciences and Nanotechnology*, *3*(3), 1043–1056. https://doi.org/10.37285/ijpsn.2010.3.3.2
24. Feng, B., Matsui, H., & Tomizawa, K. (2011). Nanoparticle-based Drug Delivery Systems for Solid Brain Tumors. *Current Nanoscience*, *7*(1), 47–54. https://doi.org/10.2174/157341311794480327
25. Attia, M. F., Anton, N., Wallyn, J., Omran, Z., & Vandamme, T. F. (2019). An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. In *Journal of Pharmacy and Pharmacology* (Vol. 71, Issue 8, pp. 1185–1198). https://doi.org/10.1111/jphp.13098
26. Yan, L., Shen, J., Wang, J., Yang, X., Dong, S., & Lu, S. (2020). Nanoparticle-Based Drug Delivery System: A Patient-Friendly Chemotherapy for Oncology. *Dose-Response*, *18*(3), 1–12. https://doi.org/10.1177/1559325820936161
27. Suri, S. S., Fenniri, H., & Singh, B. (2007). Nanotechnology-based drug delivery systems. *Journal of Occupational Medicine and Toxicology*, *2*(1), 1–6. https://doi.org/10.1186/1745-6673-2-16
28. Haider, S. (2020). *Nanoparticles : the Future of Drug Delivery*. *38*(1), 1–2.
29. Raosaheb, H. S., Mohanty, P., & Jain, A. (2019). *Solid Lipid Nanoparticles for Brain Targeting*. *9*, 911–915.
30. Suri, S. S., Fenniri, H., & Singh, B. (2007). *Journal of Occupational Medicine Nanotechnology-based drug delivery systems*. *6*, 1–6. https://doi.org/10.1186/1745-6673-2-16
31. Yogesh, S., Sumeet, D., Shailesh, G., Alpa, J., & Anil, K. (n.d.). *A comprehensive review on nanoparticle drug delivery system*.
32. Nikalje, A. P. (2015). *Nanotechnology and its Applications in Medicine*. *5*, 81–89. https://doi.org/10.4172/2161-0444.1000247

33. Welfare, F. (2019). *Guidelines for Evaluation of Nanopharmaceuticals in India Guidelines For Evaluation Of Nanopharmaceuticals In India*.
34. Silva, J., Fernandes, A. R., & Baptista, P. V. (n.d.). *Application of Nanotechnology in Drug Delivery*. 14–17.
35. Nadu, T. (n.d.). *Nanomedicine for cancer targeted drug delivery , early detection mechanism and therapeutics*.
36. Bae, Y. H., & Park, K. (2011). Targeted drug delivery to tumors: Myths, reality and possibility. *Journal of Controlled Release*, *153*(3), 198–205. https://doi.org/10.1016/j.jconrel.2011.06.001
37. Caffo, M., Cardali, S., Fazzari, E., Barresi, V., & Caruso, G. (2018). Nanoparticles drug-delivery systems and antiangiogenic approaches in the treatment of gliomas. *Glioma*, *1*(6), 183. https://doi.org/10.4103/glioma.glioma_43_18
38. Gao, H. (2016). Progress and perspectives on targeting nanoparticles for brain drug delivery. *Acta Pharmaceutica Sinica B*, *6*(4), 268–286. https://doi.org/10.1016/j.apsb.2016.05.013
39. Galstyan, A., Markman, J. L., Shatalova, E. S., Chiechi, A., Korman, A. J., Patil, R., Klymyshyn, D., Tourtellotte, W. G., Israel, L. L., Braubach, O., Ljubimov, V. A., Mashouf, L. A., Ramesh, A., Grodzinski, Z. B., Penichet, M. L., Black, K. L., Holler, E., Sun, T., Ding, H., … Ljubimova, J. Y. (2019). Blood–brain barrier permeable nano immunoconjugates induce local immune responses for glioma therapy. *Nature Communications*, *10*(1), 1–13. https://doi.org/10.1038/s41467-019-11719-3
40. Veiseh, O., Gunn, J. W., & Zhang, M. (2010). Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging. *Advanced Drug Delivery Reviews*, *62*(3), 284–304. https://doi.org/10.1016/j.addr.2009.11.002
41. He, Z., Liu, K., Byrne, H. J., Cullen, P. J., Tian, F., & Curtin, J. F. (2019). Combination Strategies for Targeted Delivery of Nanoparticles for Cancer Therapy. *Applications of Targeted Nano Drugs and Delivery Systems*, 191–219. https://doi.org/10.1016/b978-0-12-814029-1.00008-9
42. Khan, A. K., Rashid, R., Murtaza, G., & Zahra, A. (2014). Gold nanoparticles: Synthesis and applications in drug delivery. *Tropical Journal of Pharmaceutical Research*, *13*(7), 1169–1177. https://doi.org/10.4314/tjpr.v13i7.23
43. Soni, S., Ruhela, R. K., & Medhi, B. (2016). Nanomedicine in central nervous system (CNS) disorders: A present and future prospective. *Advanced Pharmaceutical Bulletin*, *6*(3), 319–335. https://doi.org/10.15171/apb.2016.044
44. Liu, Y., Shah, S., & Tan, J. (2012). Computational Modeling of Nanoparticle Targeted Drug Delivery. *Reviews in Nanoscience and Nanotechnology*, *1*(1), 66–83. https://doi.org/10.1166/rnn.2012.1014
45. Zhang, Z., Guan, J., Jiang, Z., Yang, Y., Liu, J., Hua, W., Mao, Y., Li, C., Lu, W., Qian, J., & Zhan, C. (2019). Brain-targeted drug delivery by manipulating protein corona functions. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-11593-z
46. Ochekpe, N. A., Olorunfemi, P. O., & Ngwuluka, N. C. (2009). Nanotechnology and drug delivery part 1: Background and applications. *Tropical Journal of Pharmaceutical Research*, *8*(3), 265–274. https://doi.org/10.4314/tjpr.v8i3.44546
47. Guillama Barroso, G., Narayan, M., Alvarado, M., Armendariz, I., Bernal, J., Carabaza, X., Chavez, S., Cruz, P., Escalante, V., Estorga, S., Fernandez, D., Lozano, C., Marrufo, M., Ahmad, N., Negrete, S., Olvera, K., Parada, X., Portillo, B., Ramirez, A., … Viel, S. (2020). Nanocarriers as Potential Drug Delivery Candidates for Overcoming the Blood-Brain Barrier: Challenges and Possibilities. *ACS Omega*, *5*(22), 12583–12595. https://doi.org/10.1021/acsomega.0c01592
48. Badar A, Pachera S, As, A., & Nk, L. (2019). Citation for this article: Badar A, Pachera S, Ansari AS and Lohiya NK. Nano Based Drug Delivery Systems: Present and Future Prospects. *Nanomedicine And Nanotechnology Journal*, *2*(1), 121. https://scientificliterature.org/Nanomedicine/Nanomedicine-19-121.pdf
49. Kushwaha, S. K. S., Ghoshal, S., Rai, A. K., & Singh, S. (2013). Carbon nanotubes as a novel drug delivery system for anticancer therapy: A review. *Brazilian Journal of Pharmaceutical Sciences*, *49*(4), 629–643. https://doi.org/10.1590/S1984-82502013000400002
50. Gupte, A. H., & Kathpalia, H. T. (2014). Recent advances in brain targeted drug delivery systems: A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(SUPPL. 2), 51–57.
51. Garbayo, E., Mendoza, A., & Blanco-Prieto, M. (2014). Diagnostic and Therapeutic Uses of Nanomaterials in the Brain. *Current Medicinal Chemistry*, *21*(36), 4100–4131. https://doi.org/10.2174/0929867321666140815124246

52. Agrahari, V. (2017). The exciting potential of nanotherapy in brain-tumor targeted drug delivery approaches. *Neural Regeneration Research*, *12*(2), 197–200. https://doi.org/10.4103/1673-5374.200796
53. Fallis, A. . (2013). Cancer Nanotechnology Plan 2015. *Journal of Chemical Information and Modeling*, *53*(9), 1689–1699.
54. Wang, J., Hu, X., & Xiang, D. (2018). Nanoparticle drug delivery systems: An excellent carrier for tumor peptide vaccines. *Drug Delivery*, *25*(1), 1319–1327. https://doi.org/10.1080/10717544.2018.1477857
55. Jain, K. K. (2005). Nanotechnology-based drug delivery for cancer. *Technology in Cancer Research and Treatment*, *4*(4), 407–416. https://doi.org/10.1177/153303460500400408
56. Bandyopadhyay, S. K., & Roy, S. (2018). *Nanotechnology uses Detection of Brain Tumor-A Review*. *3*(4), 115–118. https://doi.org/10.19080/GJN.2018.03.555622
57. Islam, Y., Leach, A. G., Smith, J., Pluchino, S., Coxonl, C. R., Sivakumaran, M., Downing, J., Fatokun, A. A., Teixidò, M., & Ehtezazi, T. (2020). Peptide based drug delivery systems to the brain. *Nano Express*, *1*(1), 012002. https://doi.org/10.1088/2632-959x/ab9008
58. Naqvi, S., Panghal, A., & Flora, S. J. S. (2020). Nanotechnology: A Promising Approach for Delivery of Neuroprotective Drugs. *Frontiers in Neuroscience*, *14*(June), 1–26. https://doi.org/10.3389/fnins.2020.00494
59. Viswanath, V., Rajaram, C., Fathima, S. R., & Rupa, C. (2018). *ISSN : 2320-2831 Brain targeting drug delivery system*. *7*(4), 510–520.
60. Kerkeni, L., Ruano, P., Delgado, L. L., Picco, S., Villegas, L., Tonelli, F., Merlo, M., Rigau, J., Diaz, D., & Masuelli, M. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *tourism*, 13. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics
61. Gandhi, H., Sharma, A. K., Mahant, S., & Kapoor, D. N. (2020). Recent advancements in brain tumor targeting using magnetic nanoparticles. *Therapeutic Delivery*, *11*(2), 97–112. https://doi.org/10.4155/tde-2019-0077
62. Masserini, M. (2013). Nanoparticles for Brain Drug Delivery. *ISRN Biochemistry*, *2013*, 1–18. https://doi.org/10.1155/2013/238428
63. Cerna, T., Stiborova, M., Adam, V., Kizek, R., & Eckschlager, T. (2016). Nanocarrier drugs in the treatment of brain tumors. *Journal of Cancer Metastasis and Treatment*, *2*(10), 407. https://doi.org/10.20517/2394-4722.2015.95
64. Teleanu, D. M., Chircov, C., Grumezescu, A. M., Volceanov, A., & Teleanu, R. I. (2018). Blood-brain delivery methods using nanotechnology. *Pharmaceutics*, *10*(4), 1–16. https://doi.org/10.3390/pharmaceutics10040269
65. Wei, X., Chen, X., Ying, M., & Lu, W. (2014). Brain tumor-targeted drug delivery strategies. *Acta Pharmaceutica Sinica B*, *4*(3), 193–201. https://doi.org/10.1016/j.apsb.2014.03.001
66. Singh RP, Sharma G, Sonali, et al. Effects of transferrin conjugated multi-walled carbon nanotubes in lung cancer delivery. Mater Sci Eng C Mater Biol Appl. 2016; 67: 313-325.
67. Shapira A, Livney YD, Broxterman HJ, Assaraf YG. Nanomedicine for targeted cancer therapy: towards the overcoming of drug resistance. Drug Resist Updat. 2011; 14(3): 150-163.
68. Doane TL, Burda C. The unique role of nanoparticles in nanomedicine: imaging, drug delivery and therapy. Chem Soc Rev. 2012; 41(7): 2885-2911.
69. Orringer DA, Koo YE, Chen T, et al. In vitro characterization of a targeted, dye-loaded nanodevice for intraoperative tumor delineation. Neurosurgery. 2009; 64(5): 965-971.
70. Qin L, Wang C-Z, Fan H-J et al. A dual-targeting liposome conjugated with transferrin and arginine-glycine-aspartic acid peptide for glioma-targeting therapy. Onco Lett. 2014; 8:2000-2006.
71. Sánchez-Purrà M, Ramos V, Petrenko VA et al. Double-targeted polymersomes and liposomes for multiple barrier crossing. Int J Pharm. 2016;511(2):946-956.
72. Levine RM, Kokkoli E. Dual-ligand $\alpha 5\ \beta 1$and $\alpha 6\ \beta 4$ integrin targeting enhances gene delivery and selectivity to cancer cells. J Control Release. 2017; 251:24-36.
73. Parhi P, Mohanty C, Sahoo SK. Nanotechnology-based combinational drug delivery: an emerging approach for cancer therapy. Drug Discov Today. 2012; 17(17-18): 1044-1052.
74. Chen B, Dai W, He B, et al. Current multistage drug delivery systems based on the tumor microenvironment. Theranostics. 2017; 7(3): 538-558.

75. Thomas AA, Omuro A. Current role of anti-angiogenic strategies for glioblastoma. Curr Treat Options Oncol. 2014; 15(4): 551-566.
76. Fang W, Wei Y. Upconversion nanoparticle as a theranostic agent for tumor imaging and therapy. J Innov Opt Health Sci. 2016; 9: 1630006.
77. Mitchell DA, Fecci PE, Sampson JH.Immunotherapy of malignant brain tumors. Immunol Rev. 2008; 222: 70-100.
78. Weiss T, Weller M, Roth P. Immunotherapy for glioblastoma: concepts and challenges. Curr Opin Neurol. 2015; 28(6): 639-646.
79. Stylli SS, Howes M, MacGregor L, et al. Photodynamic therapy of brain tumors: evaluation of porphyrin uptake versus clinical outcome. J Clin Neurosci. 2004; 11(6): 584-596.
80. Cheng Y, Morshed RA, Auffinger B, et al. Multifunctional nanoparticles for brain tumor imaging and therapy. Adv Drug Deliv Rev. 2014; 66: 42-57.
81. Kim SS, Harford JB, Pirollo KF, Chang EH. Effective treatment of glioblastoma requires crossing the blood-brain barrier and targeting tumors including cancer stem cells: The promise of nanomedicine. Biochem Biophys Res Commun. 2015; 468(3): 485-489.
82. Ostrom QT, Gittleman H, Farah P, et al. CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. Neuro Oncol. 2013; 15 Suppl 2: ii1-56.
83. Gao JQ, Lv Q, Li LM, et al. Glioma targeting and blood-brain barrier penetration by dual-targeting doxorubicin liposomes. Biomaterials. 2013; 34(22): 5628-5639.
84. Kabitha, KK., Rajan MS, Hegde K et al. A comprehensive review on brain tumor. J Pharm Chem Biol Sci. 2013; 3(4): 1165- 1171.
85. Laquintana V, Trapani A, Denora N, et al. New strategies to deliver anticancer drugs to brain tumors. Expert Opin Drug Deliv. 2009; 6(10): 1017-1032.
86. Shai RM, Reichardt JK, Chen TC. Pharmacogenomics of brain cancer and personalized medicine in malignant gliomas. Future Oncol. 2008; 4(4): 525-534.
87. Koo YE, Reddy GR, Bhojani M, et al. Brain cancer diagnosis and therapy with nanoplatforms. Adv Drug Deliv Rev. 2006; 58(14): 1556-1577.
88. Wei X, Chen X, Ying M, Lu W. Brain tumor-targeted drug delivery strategies. Acta Pharm Sin B. 2014; 4(3): 193-201.
89. Zong T, Mei L, Gao H, et al. Synergistic dual-ligand doxorubicin liposomes improve targeting and therapeutic efficacy of brain glioma in animals. Mol Pharm. 2014; 11(7): 2346-2357.
90. Gary-Bobo M, Hocine O, Brevet D, et al. Cancer therapy improvement with mesoporous silica nanoparticles combining targeting, drug delivery and PDT. Int J Pharm. 2012; 423(2): 509-515.
91. Mamaeva V, Sahlgren C, Lindén M. Mesoporous silica nanoparticles in medicine--recent advances. Adv Drug Deliv Rev. 2013; 65(5): 689-702.
92. Choi KY, Liu G, Lee S, Chen X. Theranostic nanoplatforms for simultaneous cancer imaging and therapy: current approaches and future perspectives. Nanoscale. 2012; 4(2): 330-342.
93. Chen NT, Cheng SH, Souris JS, et al. Theranostic applications of mesoporous silica nanoparticles and their organic/inorganic hybrids. J Mater Chem B. 2013; 1: 3128-3135.
94. Wang H, Liu X, Wang Y, et al. Doxorubicin conjugated phospholipid prodrugs as smart nanomedicine platforms for cancer therapy. J Mater Chem B. 2015; 3: 3297-3305.
95. Cheng SH, Lee CH, Chen MC, et al. Tri-functionalization of mesoporous silica nanoparticles for comprehensive cancer theranostics-the trio of imaging, targeting and therapy. J Mater Chem. 2010; 20: 6149–6157.
96. Feng Y, Panwar N, Hang Tng DJ, et al. The application of mesoporous silica nanoparticle family in cancer theranostics. Coord Chem Rev. 2016; 319: 86–109.
97. Huang X, Zhang F, Wang H, et al. Mesenchymal stem cell-based cell engineering with multifunctional mesoporous silica nanoparticles for tumor delivery. Biomaterials. 2013; 34(7): 1772-1780.
98. Wang M, Abbineni G, Clevenger A, et al. Upconversion nanoparticles: synthesis, surface modification and biological applications. Nanomedicine. 2011; 7(6): 710-729.

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REVIEW ARTICLE

A Review on Characteristics of Nano Fluids

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ABSTRACT

The heat transfer phenomenon plays an important role in the industry processes. Nano particles are very good in heat carrier. This property has been utilized in different manner by different researchers. Nano particles mixed with fluid give rise colloidal suspensions that carry much more heat as compare to pure fluid. So, the nano fluids acted as coolant in machinery where huge amount of heat generate. Again, the material from which the nano particles are formed, size of the particles and the fluid with which the nano particles are mixed matters the behavior of nanofluid and that is reviewed in this paper.

Keywords: Nano fluid, Nano particle, Heat transfer, Brownian motion, Viscous dissipation

INTRODUCTION

A fluid containing nanometer sized particles which are known as nanoparticles, is called as nanofluid. These fluids are caused due to the colloidal suspensions of nanoparticles in a base fluid. Different minerals, oxides, carbides, carbons etc. are the nanoparticles which are used in nanofluids. Water, oil, ethylene glycol are the base fluids. Nanofluids has huge applications in hybrid powered engines, vehicle thermal management, domestic refrigerator, chiller machine, heat exchanger equipment, in boiler flue gas temperature reduction etc. The concept of rheological behavior of nanofluid is used for convective heat transfer application. Nanofluids are primarily used for their enhanced thermal properties as in heat transfer equipment such as heat exchanger, electronic colling system, radiation etc. Solar collector is another application of nanofluid in which they are employed for their optical properties.

Literature review

The term nanofluid refers to fluid with suspended nanoparticles, have been introduced by Choi and Eastman [1] in 1995. Choi et. al [2] also concluded that the thermal conductivity of the base fluid is being improved by adding the nanoparticles with it. Afterwards, it was concluded by Buongiorno [3] that the improvement in the thermal conductivity happens with the occurrence of Brownian and thermophoretic diffusions inside the flow region. A numerical analysis of three-dimensional water based nanofluid over a stretching sheet was conducted by Nadeem et

al [4]. A complete study of heat transferring nanofluids was made by Buongiorno and Hu [5].Kameswaran et. al [6] have conducted a study on hydromagnetic nanofluid flow due to stretching sheet with chemical reaction, viscous dispassion and Soreteffects. They were studied two types of nanofluid like Cu-water and Ag-water. Nanoparticle volume fraction have been used by them for describing the nano-fluid flow. Radiation effect on the viscus flow of a nano fluid and heat transfer on a nonlinear stretching sheet was analyzed by Hady et.al.[7].Makinde et. al. [8] have studied numerically on the boundary layer flow made by a nano fluid due to stretching sheet. Their leading transport equations were included with Brownian motion and thermosphere. In conclusion they got that the transport of momentum, energy and concentration of nanoparticles are depends on the parameters like "Brownian motion parameter (Nb), thermophoresis parameter (Nt), Pradalt number (Pr), Lewis number(Le) and Biot number(Bi)".A study on two-dimensional steady hydrodynamic boundary layer flow of a electrically conducting nano fluid which is past over a stretching sheet with Newtonian heat has been conducted by Mahato et. al [9].

The nanofluid was viscous and Incompressible also. The set of governing transport equation with Brownian motion was solved by Spectral Relaxation Method (SRM) after making suitable transformation. They observed that the applied magnetic field take a great role to slow down the nanofluid flow whereas it increases the nanofluid temperature and nanoparticle concentration. Gireesha B.J et. al. [10] have given a numerically computation result on a steady two-dimensional stagnation point flow of a nano fluid which past over a stretching surface with magnetic field, melting effect and heat absorption. It observed that the flow pattern became changed due to the adding of nanoparticle with the base fluid. They also found that the magnetic field and temperature distribution increases with the impact of hydromagnetic field. Abbas W. [11] did a work on improvement of the thermophysical properties of an unsteady nanofluid which is past over a rotating plate in presence of ohmic, viscous dissipation and thermophoresis diffusion. In conclusion they found that the shape of the nanoparticle makes a great roll for the determination of the flow behavior. They also got that the cylindrical shape nanoparticle is better than other nanoparticle in heat transfer case. Falana A. [12] have conducted a study on the impact of thermophoresis and Brownian motion on a nonlinear permeable stretching sheet which is in the nano fluid. Shooting method with runge-kutta technique has been used for solving the governing equations. They found that there is a increasing in temperature with increasing in thermophoresis parameter or Brownian motion parameter. N Bozorgan [13] have conducted a numerical study on the application of CuO-water nanofluid in a radiator of a Chevrolet Suburban diesel engine under turbulent flow condition. There was a study on the heat, mass transfer and magnetic field of a mixed copper-water nanofluid past by an inclined plate with porous medium conducted by Md Nasir Uddin et. al. [14].

The transformation equation of the nanofluid included with chosen boundary conditions which are solved by "Runge-Kutta method of sixth-order" together with "Nachtsheim and Swigert technique". From their study they concluded that, for increasing of magnetic parameter, local Nustle number decreases but the skin friction coefficient increases. Also, skin friction parameter increases with increasing of permeability parameter. In a study M. Wahiduzzaman et. al. [15] have analyzed about the radiative heat transfer and viscous dispersion in a nanofluid with the impact of magnetic field over a rotating stretching surface. They considered steady laminar boundary layer flow in their study. They solved the governing equations with the help of "Runge-Kutta integration process of sixth-order" together with shooting method. In the result the different parameters like velocity, temperature and distribution parameter are shown graphically. U. Rashid et. al. [16] have examined the effect of nanoparticle shape on Al_2O_3-water nanofluid and heat transfer over a stretching sheet which is nonlinear and radical in nature, in presence of thermal radiation and magnetic field. They used bvp4c method for solving their governing equations. It is concluded that the rate of heat transfer in lamina nanoparticle is more than the other shaped nanoparticle and a large temperature distribution occurred in lamina shaped nanoparticle as compared with other shaped nanoparticles. Farhad A Abbassi et. Al. [17] have studied the laminar forced convection of CuO nanofluid. A Eulerian two fluid model is considered for the nanofluid. The governing mass and energy equation has been solved by finite volume method. From the investigation it had observed that the Nusselt number increases when the expansion ratio has been decreases as well as increasing in Reynolds number. The two-phase models are used efficiently instead of single-phase model. Mohammed J Uddin et. Al. [18] have analysed a numerical conclusion of

steady 2-D laminar boundary layer flow of a Newtonian nanofluid which was electrically conducting. The given nanofluid was on a solid vertical plate. The governing flow problem equations are solved by "Runge-kutta Fehlberg fourth-fifth order method". As a conclusion they found that when the Newtonian heating parameter rises, the rate of heat and mass transfer increases and the velocity, temperature distribution increases also. P Durga Prasad et. Al. [19] have studied on the effect of radiation, absorption and chemical reaction on MHD free convective heat and mass transfer flow of a nanofluid.

The diffusion theorem parameter absorptive enhance the velocity and temperature and skin friction also. The analytical solution of boundary layer flow equations were concluded as oscillatory type and then solved by perturbation technique. They have found on result that the decreasing of species concentration gives the increasing of suction parameter and chemical reaction. There is an effect of magnetic field on skin friction coefficient. Siva Reddy Sheri et. al. [20] have considered a study on heat transfer in MHD free convenient flow by the help of nanofluid. The problem was also considered by nanofluid volume fraction model by taking water based nanofluids which contains aluminium and oxide and copper. The governing equations are solved by the help of finite volume method. In conclusion they found that the parameters like permeability parameter (K), thermal radiation (F) &Eckert number (Ec) were accelerating the velocity of the nanofluid where as the other parameters like dimensionless magnetic field parameter (M), dimensional rotational parameter(R), suction parameter (S), non-dimensional heat source parameter (Q) & Prandtl number(Pr) were decreasing the velocity of nanofluid. C.S.K Raju et. al. [21] have conducted a study on the impact of space and heat generation on 3D MHD nanofluid flow which past on a stretching sheet which is permeable and nonlinear. The governing equations are solved by the help of Matlab bvp4c method. They found the conclusion that for increase of magnetic field, the friction factor and rate of heat transfer decrease. N Sandeep et. al. [22] have proposed a study about a MHD unsteady radiative flow and heat transfer of a dusty nano fluid which passed over a permeable stretching surface. They solved the governing equations by using shooting method. From their study they revealed that increase of volume fraction made with increase of friction factor and heat transfer rate.

CONCLUSION

After reviewing above mentioned papers, it is determined that the research works on the nanofluid is a multidirectional task. After reviewing many papers of nanofluid, it is observed that the role of different parameters like rotational parameter, magnetic field parameter, "Prandtl number", Reynolds number, permeability parameter etc. on velocity, temperature and thermal radiation are studied. It is clear that the thermal conductivity character of nanofluid has a huge application at every corner of the world in fluid dynamics applications.

REFERENCES

1. S. U. S. Choi and J. A. Eastman, "Enhancing thermal conductivity of fluids with nanoparticles", *The Proceedings of the 1995 ASME International Mechanical Engineering Congress and Exposition*, San Francisco, USA, ASME, FED 231/MD 66, pp. 99-105, 1995.
2. S. U. S. Choi, Z. G. Zhang, W. Yu, F. E. Lockwood and E. A. Grulke, "Anomalously thermal conductivity enhancement in nanotube suspensions", *Appl. Phys. Lett.*, Vol. 79, pp. 2252-2254, 2001.
3. J. Buongiorno. "Convective transport in nanofluids". *J. Heat Transf.*, pp. 240- 250,2006.
4. S. Nadeem, R. UlHaq, and Z. H. Khan. "Heat transfer analysis of water-based nanofluid over an exponentially stretching sheet",*Alexandria Eng. Journal*., pp. 219-224,2014.
5. J.Buongiorno and W. Hu. "Nanofluid coolants for advanced nuclear power plants "*Proceedings of ICAPP'05*, Seoul, pp. 5705, 2005.
6. Kameswaran, P. K., M. Narayana, P. Sibanda and P. V. S. N. Murthy "Hydromagnetic nanofluid flow due to a stretching or shrinking sheet with viscous dissipation and chemical reaction effect". *Int. J. Heat Mass Transfer*, pp. 7587–7595, 2012.

7. Hady, F. M., F. S. Ibrahim, S. M. Abdel-Gaied and M. R. Eid "Radiation effect on viscous flow of a nanofluid and heat transfer over a nonlinearly stretching sheet", *Nanoscale Research Letters*, pp. 229, 2012 .
8. Makinde, O. D. and A. Aziz, "Boundary layer flow of a nanofluid past a stretching sheet with a convective boundary condition", *Int. J. Thermal Sci*., pp. 1326–1332, 2011.
9. B. K. Mahatha, R. Nandkeolyar, G. K. Mahato and P. Sibanda, "Dissipative Effects in Hydromagnetic Boundary Layer Nanofluid Flow past a Stretching Sheet with Newtonian Heating", *Journal of Applied Fluid Mechanics*,pp. 1977-1989, 2016.
10. B.J. Gireesha, B. Mahanthesh, I.S. Shivakumara, K.M. Eshwarappa, "Melting heat transfer in boundary layer stagnation-point flow of nanofluid toward a stretching sheet with induced magnetic field", *Eng. Sci. Tech. Int. Journal,* pp. 313–321, 2016.
11. W. Abbas and M. M. Magdy, "Heat and Mass Transfer Analysis of Nanofluid Flow Based on Cu, Al2O3, and TiO2 over a Moving Rotating Plate and Impact of Various Nanoparticle Shapes", *Hindawi Mathematical Problems in Engineering*, 2020.
12. Falana, O. A. Ojewale, T. B. Adeboje, "Effect of Brownian Motion and Thermophoresis on a Nonlinearly Stretching Permeable Sheet in a Nanofluid", *Advances in Nanoparticles*, pp.123-134, 2016.
13. N. Bozorgan, K. Krishnakumar, N Bozorgan, "Numerical Study on Application of CuO-Water Nanofluid in Automotive Diesel Engine Radiator", *Modern Mechanical Engineering*, pp. 130-136, 2012
14. Md. Nasir Uddin, Md. Abdul Alim, Md. Mustafizur Rahman, "MHD Effects on Mixed Convective Nanofluid Flow with Viscous Dissipation in Surrounding Porous Medium", *Journal of Applied Mathematics and Physics*, pp. 968-982, 2019.
15. M. Wahiduzzaman, Md. Shakhaoath Khan, P. Biswas, Ifsana Karim, M. S. Uddin, "Viscous Dissipation and Radiation Effects on MHD Boundary Layer Flow of a Nanofluid Past a Rotating Stretching Sheet", *Applied Mathematics*, pp. 547-567, 2015.
16. Umair Rashid, Adnan Ibrahim, "Impacts of Nanoparticle Shape on Al2O3-Water Nanofluid Flow and Heat Transfer over a Non-Linear Radically Stretching Sheet", *Advances in Nanoparticles*, pp. 23-39, 2020
17. Farhad A. Abbassi, Mohsen Nazari, Mohammad Mohsen Shahmardan, "Numerical Study of Heat Transfer and Flow Bifurcation of CuO Nanofluid in Sudden Expansion Microchannel Using Two-Phase Model", *Modern Mechanical Engineering*, pp. 57-72, 2017.
18. Mohammed J. Uddin, Waqar A. Khan , Ahmed I. Ismail, "MHD Free Convective Boundary Layer Flow of a Nanofluid past a Flat Vertical Plate with Newtonian Heating Boundary Condition", *PLoSOne*, https://doi.org/10.1371/journal.pone.0049499, 2012.
19. P. Durga Prasad, R.V.M.S.S, Kiran Kumar, S.V.K. Varma, "Heat and mass transfer analysis for the MHD flow of nanofluid with radiation absorption", *Ain Shams Engineering Journal*pp. 801–813, 2018
20. Siva Reddy Sheri, Thirupathi Thumma, "Numerical study of heat transfer enhancement in MHD free convection flow over vertical plate utilizing nanofluids", *Ain Shams Engineering Journal,* pp. 1169–1180, 2018.
21. C.S.K. Raju, N. Sandeep, M. Jayachandra Babu, V. Sugunamma, "Dual solutions for three-dimensional MHD flow of a nanofluid over a nonlinearly permeable stretching sheet", *Alexandria Engineering Journal*, pp. 151-162, 2016.
22. N. Sandeep, C. Sulochana, B. Rushi Kumar, "Unsteady MHD radiative flow and heat transfer of a dusty nanofluid over an exponentially stretching surface", *Engineering Science and Technology, An International Journal*, pp. 227-240, 2016.

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RESEARCH ARTICLE

δĝ^- Closed Sets in Topological Spaces

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ABSTRACT

In this paper is to present a new class of sets called δĝ^-closed sets in topological spaces and we study some of its an essential properties. Become aware of this class of sets lies between the class of δ-closed sets and the class of δω-closed sets.

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Keywords: δg-closed sets, δω-closed set s, δĝ^-closed sets and δĝ^-closed sets.

INTRODUCTION

Levine **[10, 11]** was introduced by the notion of semi-open sets and the notion of g-closed sets and investigated its fundamental properties. This notion was shown to be productive and very helpful. Velicko **[20]** was introduced by the notion of δ-closed sets and it is well known that the collection of all δ-closed sets of a topological space forms a topology and is denoted by τ_δ. Dontchev and Maki **[7]** was introduced by the notion of δ-generalized closed sets. In this paper is to present a new class of sets called δĝ^-closed sets in topological spaces and we study some of its an essential properties. Become aware of this class of sets lies between the class of δ-closed sets and the class of δω-closed sets.

Preliminaries

Throughout this paper (X, τ) and (Y, σ) (or X and Y) stand for topological spaces on which no separation axioms are assumed unless otherwise mentioned. For a subset A of a space (X, τ), cl(A), int(A) and A^c or X|A denote the closure of A, the interior of A and the complement of A, respectively.

We recall the following definitions which are useful in the sequel.

Definition 2.1. A subset A of a space (X, τ) is called

1. semi-open set [11] if $A \subseteq cl(int(A))$.
2. preopen set [13] if $A \subseteq int(cl(A))$.
3. α-open set [14] if $A \subseteq int(cl(int(A)))$.
4. β-open set [1] (= semi-preopen set [2]) if $A \subseteq cl(int(cl(A)))$.
5. regular open set [17] if $A = int(cl(A))$.

The complements of the above mentioned open sets are called their respective closed sets.

Definition 2.2. [20] A point x of a space X is called a δ-adherent point of a subset A of X if $int(cl(U)) \cap A \neq \varphi$, for every open set U containing x. The set of all δ-adherent points of A is called the δ-closure of A and is denoted by $cl_\delta(A)$. A subset A of a space X is called δ-closed if and only if $A = cl_\delta(A)$. The complement of a δ-closed set is called δ-open. Similarly, the δ-interior of a set A in X, written $int_\delta(A)$, consists of those points x of A such that for some regularly open set U containing x, $U \subseteq A$. A set A is δ-open if and only if $A = int_\delta(A)$, or equivalently, $X \setminus A$ is δ-closed.

The family of all δ-open subsets of (X, τ) forms a topology on X and is denoted by τ_δ. Since the intersection of two regular open sets is regular open, the collection of all regular open sets forms a base for a coarser topology τ_s than the original one τ. The family τ_s is the so-called semi-regularization of τ. A topological space (X, τ) is called semi-regular if $\tau = \tau_s$.

Definition 2.3. A subset A of a topological space (X, τ) is called

1. A generalized closed (briefly, g-closed) set **[10]** if $cl(A) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ).
2. A generalized semi-closed (briefly, gs-closed) set **[3]** if $scl(A) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ).
3. A semi-generalized closed (briefly, sg-closed) set **[4]** if $scl(A) \subseteq U$ whenever $A \subseteq U$ and U is semi-open in (X, τ). The complement of a sg-closed set is called sg-open set.
4. An α-generalized closed (briefly, αg-closed) set **[12]** if $\alpha cl(A) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ).
5. A generalized semi-preclosed (briefly, gsp-closed) set **[6]** if $spcl(A) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ).
6. A generalized preclosed (briefly, gp-closed) set **[15]** if $pcl(A) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ).
7. A $\hat{g}$-closed set **[18]** (= ω-closed set **[16]**) if $cl(A) \subseteq U$ whenever $A \subseteq U$ and U is semi-open in (X, τ).
8. A $\delta\hat{g}$^-closed set **[9]** if $cl(A) \subseteq U$ whenever $A \subseteq U$ and U is sg-open in (X, τ).
9. A ψ-closed set **[19]** if $scl(A) \subseteq U$ whenever $A \subseteq U$ and U is sg-open in (X, τ).
10. A δ-generalized closed (briefly, δg-closed) set **[7]** if $cl_\delta(A) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ).
11. A $\delta\omega$-closed set **[8]** if $cl_\delta(A) \subseteq U$ whenever $A \subseteq U$ and U is semi-open in (X, τ).

Remark 2.4. The collection of all δg-closed (resp. $\delta\hat{g}$^-closed, $\delta\omega$-closed, sg-closed, ψ-closed, ω-closed, g-closed, δ-closed, α-closed, semi-closed) sets of X is denoted by $\delta GC(X)$ (resp. $\delta\hat{G}^{\wedge}C(X)$, $\delta\omega C(X)$, $SGC(X)$, $\psi C(X)$, $\omega C(X)$, $GC(X)$, $\delta C(X)$, $\alpha C(X)$, $SC(X)$). We denote the power set of X by P(X).

Remark 2.5. [16]

(1) Every δ-closed set is δg-closed.

(2) δg-closed sets and ω-closed sets are independent.

Definition 2.6. [7] The intersection of all sg-open subsets of (X, τ) containing A is called the sg-kernel of A and is denoted by sg-ker(A).

Definition 2.7. [5] (X, τ) is semi-regular if and only if $\tau_\delta = \tau$.

Definition 2.8. [5] A space (X, τ) is said to be subweakly T_2 if $cl_\delta(\{x\}) = cl(\{x\})$ for each $x \in X$.

Proposition 2.9. [7] Let (X, τ) be a space. If $A \subseteq X$ is preopen then $cl(A) = \alpha cl(A) = cl_\delta(A)$.

Definition 2.10. [10] A space (X, τ) is called $T_{1/2}$-space if every g-closed set in X is closed in X.

Lemma 2.11. [7] In any space, a singleton is δ-open if and only if it is regular open.

Definition 2.12. [7] A space (X, τ) is called an almost weakly Hausdorff if it is $T_{1/2}$ semi-regularization.

Definition 2.13. [7] A space (X, τ) is called an R_1-space if every two different points with distinct closures have disjoint neighborhoods.

Corollary 2.14. [7] In an almost weakly Hausdorff space (X, τ) the g-closed sets of (X, τ_s) are δ-closed in (X, τ) and thus δg-closed in (X, τ).

Theorem 2.15. [7] The intersection of a δg-closed set and a δ-closed set is always δg-closed.

ON δĝ^-CLOSED SETS

Definition 3.1. A subset A of a space (X, τ) is said to be a δĝ^-closed set if $cl_\delta(A) \subseteq U$ whenever $A \subseteq U$ and U is sg-open. The complement of δĝ^-closed set is called a δĝ^-open. The collection of all δĝ^-closed sets of X is denoted by δĜ^C(X).

Proposition 3.2. In a space (X, τ), each δ-closed set is δĝ^-closed.

Proof. Let A be a δ-closed set and B be any sg-open set containing A. Since A is δ-closed, $cl_\delta(A) = A$ for every subset A of X. Therefore $cl_\delta(A) \subseteq B$ and hence A is δĝ^-closed.

Remark 3.3. The following example show that converse of Proposition 3.2 is not true.

Example 3.4. Let X = {1, 2, 3} with τ = {ϕ, {1}, X}. Then δĜ^C(X) = {ϕ, {2, 3}, X} and δC(X) = {ϕ,X}. In the space (X,τ), then A = {2,3} is a δĝ^-closed set but not δ-closed.

Proposition 3.5. In a space (X, τ), every δĝ^-closed set is δg-closed.

Proof. Let A be a δĝ^-closed set and B be any open set containing A. Since every open set is sg-open and A is δĝ^-closed, $cl_\delta(A) \subseteq B$. Therefore $cl_\delta(A) \subseteq B$ and B is open. Hence A is δg-closed.

Remark 3.6. The following example show that converse of Proposition 3.5 is not true.

Example 3.7. In example 3.2, we have δĜ^C(X) = {ϕ, {2, 3}, X} and δGC(X) = {ϕ, {2}, {3}, {1, 2}, {1, 3}, {2, 3}, X}. In the space (X, τ), then A = {1, 3} is a δg-closed set but not δĝ^-closed.

Proposition 3.8. In a space (X, τ), every δĝ^-closed set is δω-closed.

Proof. Let A be a δĝ^-closed set and B be any semi-open set containing A. Since every semi-open set is sg-open and A is δĝ^-closed, $cl_\delta(A) \subseteq B$. Hence A is δω-closed.

Remark 3.9. The following example show that converse of Proposition 3.8 is not true.

Example 3.10. Let X = {1, 2, 3} with τ = {ϕ, {1}, {2, 3}, X}. We have δĜ^C(X) = {ϕ, {1}, {2, 3}, X} and δωC(X) = P(X). In the space (X, τ), then A = {1, 2} is a δω- closed but not δĝ^-closed.

Proposition 3.11. In a space (X, τ), every δĝ^-closed set is g-closed.

Proof. Let A be a δĝ^-closed set and B be any open set containing A. Since every open set is sg-open and A is δĝ^-closed, $cl_\delta(A) \subseteq B$. Since $cl(A) \subseteq cl_\delta(A) \subseteq B$, $cl(A) \subseteq B$ and hence A is g-closed.

Remark 3.12. The following example show that converse of Proposition 3.11 is not true.

Example 3.13. In Example 3.2, we have δĜ^C(X) = {ϕ, {2, 3}, X} and GC(X) = {ϕ, {2}, {3}, {1, 2}, {1, 3}, {2, 3}, X}. In the space (X, τ), then A = {1, 2} is a g-closed set but not δĝ^-closed.

Proposition 3.14. In a space (X, τ), every δĝ^-closed set is ω-closed.

Proof. Let A be a δĝ^-closed set and B be any semi-open set containing A. Since every semi-open set is sg-open and A is δĝ^-closed, $cl_\delta(A) \subseteq B$. Since $cl(A) \subseteq cl_\delta(A) \subseteq B$, $cl(A) \subseteq B$ and hence A is ω-closed.

Remark 3.15. The following example show that converse of Proposition 3.14 is not true.

Example 3.16. Let X = {1, 2, 3} with τ = {ϕ, {1}, {1, 2}, X}. We have δĜ^C(X) = {ϕ, {2, 3}, X} and ωC(X) = {ϕ, {3}, {2, 3}, X}. In the space (X, τ), then A = {3} is a ω-closed set but not δĝ^-closed.

Proposition 3.17. In a space (X, τ), every δĝ^- closed set is ĝ^-closed.

Proof. Let A be a δĝ^-closed set and B be any sg-open set containing A. Since $cl(A) \subseteq cl_\delta(A) \subseteq B$ and hence A is ĝ^-closed.

Remark 3.18. The following example show that converse of Proposition 3.17 is not true.

Example 3.19. In Example 3.16, we have $\delta\hat{G}C(X) = \{\phi, \{2, 3\}, X\}$ and $\delta\hat{G}^{\wedge}C(X) = \{\phi, \{3\}, \{2, 3\}, X\}$. In the space (X, τ), then $A = \{3\}$ is a $\hat{g}^{\wedge}$-closed but not $\delta\hat{g}^{\wedge}$-closed.

Proposition 3.20. In a space (X, τ), every $\delta\hat{g}^{\wedge}$-closed set is sg-closed.

Proof. Let A be a $\delta\hat{g}^{\wedge}$-closed set and B be any semi-open set containing A. Since every semi-open set is sg-open, $cl_{\delta}(A) \subseteq B$. Since $scl(A) \subseteq cl_{\delta}(A) \subseteq B$, $scl(A) \subseteq B$ and hence A is sg-closed.

Remark 3.21. The following example show that converse of Proposition 3.20 is not true.

Example 3.22. In Example 3.8, we have $\delta\hat{G}^{\wedge}C(X) = \{\phi, \{1\}, \{2, 3\}, X\}$ and $SGC(X) = P(X)$. In the space (X, τ), then $A = \{1, 2\}$ is a sg-closed but not $\delta\hat{g}^{\wedge}$-closed.

Proposition 3.23. In a space (X, τ), every $\delta\hat{g}^{\wedge}$-closed set is ψ-closed.

Proof. It is true from the fact that $scl(A) \subseteq cl_{\delta}(A)$ for every subset A of (X, τ).

Remark 3.24. The following example show that converse of Proposition 3.23 not true.

Example 3.25. In Example 3.4, we have $\delta\hat{G}^{\wedge}C(X) = \{\phi, \{2, 3\}, X\}$ and $\psi C(X) = \{\phi, \{2\}, \{3\}, \{2, 3\}, X\}$. In the space (X, τ), then $A = \{b\}$ is a ψ-closed but not $\delta\hat{g}^{\wedge}$-closed.

Remark 3.26. In a space (X, τ), the concepts of $\delta\hat{g}$-closed is independent of closed, semi-closed and α-closed of each other.

Example 3.27. In Example 3.4, we have $\delta\hat{G}^{\wedge}C(X) = \{\phi, \{2, 3\}, X\}$ and $\alpha C(X) = SC(X) = \{\phi, \{2\}, \{3\}, \{2, 3\}, X\}$. In the space (X, τ), then $A = \{2\}$ is an α-closed as well as semi-closed but it is not $\delta\hat{g}$-closed.

Example 3.28. Let $X = \{1, 2, 3\}$ with $\tau = \{\phi, \{1, 2\}, X\}$. We have $\delta\hat{G}^{\wedge}C(X) = \{\phi, \{3\}, \{1, 3\}, \{2, 3\}, X\}$ and $\alpha C(X) = SC(X) = \{\phi, \{3\}, X\}$. In the space (X, τ), then $A = \{1, 3\}$ is $\delta\hat{g}^{\wedge}$-closed but it is neither α-closed nor semi-closed.

Example 3.29. (1) In Example 3.16, then $\{3\}$ is a closed set but not $\delta\hat{g}^{\wedge}$-closed.(2) In Example 3.28, then $\{2, 3\}$ is a $\delta\hat{g}^{\wedge}$-closed set but not closed.

Remark 3.30. In a space (X, τ), the following example show that concepts of $\delta\omega$-closed sets and the concepts of $\hat{g}^{\wedge}$-closed sets are independent of each other.

Example 3.31. (1) In Example 3.10, then $\{1, 2\}$ is a $\delta\omega$-closed set but not $\hat{g}^{\wedge}$-closed.(2) In Example 3.16, then $\{3\}$ is a $\hat{g}^{\wedge}$-closed but not $\delta\omega$-closed.

Remark 3.32. From the above consultation are shown in the following diagram.

MORE PROPERTIES OF $\delta\hat{g}^{\wedge}$-CLOSED SETS

Theorem 4.1. A subset A of (X, τ) is $\delta\hat{g}^{\wedge}$-closed $\Longleftrightarrow cl_{\delta}(A) \subseteq$ sg-ker(A).

Proof. Suppose that A is $\delta\hat{g}^{\wedge}$-closed. Then $cl_{\delta}(A) \subseteq U$ whenever $A \subseteq U$ and U is sg-open. Let $x \in cl_{\delta}(A)$. If $x \in$ sg-ker(A), then there is a sg-open set U containing A such that $x \in U$. Since U is a sg-open set containing A, we have $x \in cl_{\delta}(A)$ and this is a contradiction. Conversely, let $cl_{\delta}(A) \subseteq$ sg-ker(A). If U is any sg-open set containing A, then $cl_{\delta}(A) \subseteq$ sg-ker(A) $\subseteq U$. Therefore, A is $\delta\hat{g}$-closed.

Remark 4.2. In a space (X, τ), if A is $\delta\hat{g}^{\wedge}$-closed and B is δ-closed, then $A \cap B$ is a δg-closed set.

Proposition 4.3. If a set A is $\delta\hat{g}^{\wedge}$-closed in (X, τ) then $cl_{\delta}(A) - A$ contains no nonempty sg-closed set in (X, τ).

Proof. Suppose that A is $\delta\hat{g}^{\wedge}$-closed. Let B be a sg-closed subset of $cl_{\delta}(A)-A$. Then $A \subseteq B^{c}$. Therefore $cl_{\delta}(A) \subseteq B^{c}$. Consequently, $B \subseteq (cl_{\delta}(A))^{c}$. We already have $B \subseteq cl_{\delta}(A)$. Thus $B \subseteq cl_{\delta}(A) \cap (cl_{\delta}(A))^{c}$ and B is empty.

Remark 4.4. The following example show that converse of Proposition 4.3 is not true.

Example 4.5. In Example 3.4. We have $A = \{2\}$ then $cl_{\delta}(A) - A$ contains no non-empty sg-closed set in (X, τ). But A is not $\delta\hat{g}^{\wedge}$-closed.

Theorem 4.6. Let $A \subseteq B \subseteq X$ and suppose that A is $\delta\hat{g}^{\wedge}$-closed in X. Then A is $\delta\hat{g}^{\wedge}$-closed relative to Y.

Proof. Let $A \subseteq B \cap C$, where C is sg-open in X. Then $A \subseteq C$ and hence $cl_{\delta}(A) \subseteq C$. This implies that $B \cap cl_{\delta}(A) \subseteq B \cap C$. Thus A is $\delta\hat{g}^{\wedge}$-closed relative to B.

Theorem 4.7. In a space (X, τ), if A is a sg-open and $\delta\hat{g}^{\wedge}$-closed then A is δ-closed.

Proof. Since A is sg-open and $\delta\hat{g}$-closed, $cl_\delta(A) \subseteq A$ and hence A is δ-closed in (X, τ).

Theorem 4.8. Let A be a preopen subset of a topological space (X, τ). Then the following statements are equivalent.

(1) A is $\delta\hat{g}$-closed.
(2) A is $\delta\omega$-closed (or $\hat{g}$-closed).
(3) A is δg-closed (or ω-closed).
(4) A is g-closed.
(5) A is αg-closed.

Proof. (1) $\Rightarrow$ (2) $\Rightarrow$ (3) $\Rightarrow$ (4) $\Rightarrow$ (5). It is obvious from Remark 3.32.

(5) $\Rightarrow$ (1). It follows from Proposition 2.9. Recall that a partition space **[7]** is a topological space where every open set is closed.

Corollary 4.9. Let A be a subset of the partition space (X, τ). Then the following statements are equivalent.

(1) A is $\delta\hat{g}$-closed.
(2) A is $\delta\omega$-closed (or $\hat{g}$-closed).
(3) A is δg-closed (or ω-closed).
(4) A is g-closed.
(5) A is αg-closed.

Proof. A topological space is a partition space if and only if every subset is preopen. Thus the claim follows straight from Theorem 4.8.

Theorem 4.10. For a singleton subset A of a subweakly T_2-space (X, τ) is A is $\delta\hat{g}$-closed iff A is $\hat{g}$-closed.

Proof. Follows from Proposition 3.17.

Conversely, note that in the subweakly T_2-spaces, the concepts of closure and δ-closure coincide for singleton sets by Definition 2.8.

Theorem 4.11. For a subset A of a topological space (X, τ), the following statements are equivalent.

(1) A is clopen.
(2) A is $\delta\hat{g}$-closed, preopen and semi-closed.
(3) A is $\delta\omega$-closed, preopen and semi-closed.
(4) A is $\delta\hat{g}$-closed and (regular) open.
(5) A is $\delta\omega$-closed and (regular) open.
(6) A is αg-closed and (regular) open.

Proof. (1) $\Rightarrow$ (2) $\Rightarrow$ (3) $\Rightarrow$ (4) $\Rightarrow$ (5) $\Rightarrow$ (6) are obvious.

(6) $\Rightarrow$ (1). It follows from Theorem 3.13 **[7].**

Definition 4.12. A topological space (X, τ) is said to be a locally sg-δ-indiscrete space if every sg-open set is δ-closed.

Theorem 4.13. A topological space (X, τ) is X is locally sg-δ-indiscrete $\Longleftrightarrow$ every subset of X is $\delta\hat{g}$-closed.

Proof. Let $A \subseteq U$ where U is sg-open in X and A is an arbitrary subset of X. Since X is locally sg-δ-indiscrete, then U is δ-closed. We have $cl_\delta(A) \subseteq cl_\delta(U) = U$. Thus A is $\delta\hat{g}$-closed.

Conversely, if $U \subseteq X$ is sg-open, then $cl_\delta(U) \subseteq U$ or equivalently U is δ-closed. Hence X is locally sg-δ-indiscrete.

Theorem 4.14. In an almost weakly Hausdorff space (X, τ) the g-closed sets of (X, τ_s) are δ-closed in (X, τ) and hence $\delta\hat{g}$-closed in (X, τ).

Proof. Let $A \subseteq X$ be g-closed subset of (X, τ_s). Let $x \in cl_\delta(A)$. If $\{x\}$ is δ-open, then $x \in A$. If not, then $X - \{x\}$ is δ-open, since X is almost weakly Hausdorff. Assume that $x \in A$. Since A is g-closed in (X, τ_s), then $cl_\delta(A) \subseteq X - \{x\}$. i.e. $x \in cl_\delta(A)$. By contradiction $x \in A$. Thus $cl_\delta(A) = A$ or equivalently A is δ-closed and hence $\delta\hat{g}$-closed in (X, τ).

Theorem 4.15. A compact subset A of R_1 topological space (X, τ) is $\delta\hat{g}$-closed set iff $\hat{g}$-closed set.

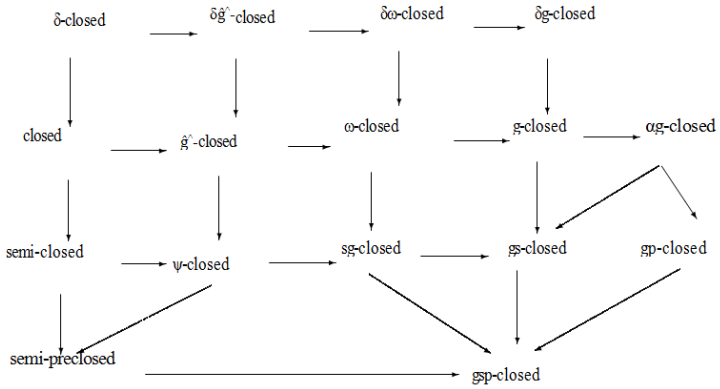
Proof. It is clear. Conversely, note that in R_1 spaces the concepts of closure and δ-closure coincide for compact sets. Thus the rest of the proof is obvious.

Theorem 4.16. In a space (X, τ), if finite union of $\delta\hat{g}$-closed sets then $\delta\hat{g}$-closed.

Proof. Let $A, B \subseteq X$ be $\delta\hat{g}$-closed and let $A \cup B \subseteq U$ where U is sg-open. Then $cl_\delta(A \cup B) = cl_\delta(A) \cup cl_\delta(B) \subseteq U$. Thus $A \cup B$ is $\delta\hat{g}$-closed.

REFERENCES

1. Abd El-Monsef, M. E., El-Deeb, S. N. and Mahmoud, R. A.: β-open sets and β-continuous mappings, Bull. Fac. Sci. Assiut Univ., 12(1983), 77-90.
2. Andrijevic, D.: Semi-preopen sets, Mat. Vesnik, 38(1986), 24-32.
3. Arya, S. P. and Nour, T. M.: Characterizations of s-normal spaces, Indian J. Pure Appl. Math.,21(8)(1990), 717-719.
4. Bhattacharyya, P. and Lahiri, B. K.: Semi-generalized closed sets in topology, Indian J. Math.,29(3)(1987), 375-382.
5. Cao, J., Ganster, M., Reilly, I. and Steiner, M.: δ-closure, θ-closure and generalized closed sets, Applied General Topology, Universidad Politecnica de Valencia, 6(1)(2005), 79-86.
6. Dontchev, J.: On generalizing semi-preopen sets, Mem. Fac. Sci. Kochi Univ. Ser. A. Math., 16(1995),35-48.
7. Dontchev, J. and Ganster, M.: On δ-generalized closed sets and $T_{3/4}$-spaces, Mem. Fac. Sci. Kochi Univ. (Math)., 17(1996), 15-31.
8. Ganesan, S., Ravi, O., Santhana, C. M. and Antony Rex Rodrigo, J.: δω-closed sets in topological spaces, Archimedes Journal of Mathematics, to appear.
9. Garg, M., Agarwal, S., and Goal, C. K.: On ĝ^-closed sets in topological spaces, Acta Ciencia Indica, XXXIII(4)M(2007), 1643-1652.
10. Levine, N.: Generalized closed sets in topology, Rend. Circ. Math. Palermo, 19(2)(1970), 89-96.
11. Levine, N.: Semi-open sets and semi-continuity in topological spaces, Amer. Math. Monthly, 70(1963), 36-41.
12. Maki, H., Devi, R. and Balachandran, K.: Associated topologies of generalized α-closed sets and α-generalized closed sets, Mem. Fac. Sci. Kochi. Univ. Ser. A. Math., 15(1994), 51-63.
13. Mashhour, A. S., Abd El-Monsef, M. E. and El-Deeb, S. N.: On precontinuous and weak pre continuous mappings, Proc. Math. and Phys. Soc. Egypt, 53(1982), 47-53.
14. Njastad, O.: On some classes of nearly open sets, Pacific J. Math., 15(1965), 961-970.
15. Noiri, T., Maki, H. and Umehara, J.: Generalized preclosed functions, Mem. Fac. Sci. Kochi Univ.Math., 19(1998), 13-20.
16. Sheik John, M.: A study on generalizations of closed sets and continuous maps in topological and bitopological spaces, Ph.D Thesis, Bharathiar University, Coimbatore, September 2002.
17. Stone, M. H.: Application of the theory of Boolean rings to general topology, Trans. Amer. Math. Soc., 41(1937), 375-381.
18. Veera Kumar, M. K. R. S.: ĝ-closed sets in topological spaces, Bull. Allahabad Math. Soc., 18(2003), 99-112.
19. Veera Kumar, M. K. R. S.: Between semi-closed sets and semi pre-closed sets, Rend Istit Mat. Univ. Trieste, Vol XXXII (2000), 25-41.
20. Velicko, N. V.: H-closed topological spaces, Amer. Math. Soc. Transl., 78(1968), 103-118.



None of the above implications are reversible.

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REVIEW ARTICLE

Dyes and Removal Strategy – A Review

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ABSTRACT

Water is one of the most valuable resources on planet earth. It is the lifeline for almost all living things on earth. Although the fact is widely recognised, pollution of water resources is a common occurrence. Water pollution is one of the major environmental problems that cause severe threat to living organisms. Increased population, industrialization and urbanisation are responsible for environmental pollution. Industrialization of numerous sectors such as food, pharmaceuticals, leather, textile, cosmetics, paper and printing, etc. Utilize dye compounds to colour their end products. Water is the main component used in all these types of industries. Water used for different processes is not completely utilized and it is discharged as waste water. Among these industries, the textile industry is the principal contributor of wastewater effluents due to the high utilization of water during dyeing, washing and finishing processes.

Keywords: Pollution, Wastewater, Treatment, Adsorption

INTRODUCTION

Textile Industry

Textile industry is one of the biggest consumers of potable water as well as chemicals used during textile processing stages. Dyeing and finishing staged are the major producers of waste water with complex characteristics. At the global level, textile industry represents an important factor of economic growth for many countries. Indian textile industry is one of the oldest and largest industries in the world and employed many people directly or indirectly [1]. The textile units are scattered all over India, out of 21076 units, Tamilnadu alone has 52285 units. The textile industry has a $1 trillion worldwide business. There are more than 8000 chemical products associated with the dyeing process, while over 100000 commercially available dyes exist with over 7x 10 power 5 metric tons of dyestuff produced annually. It is estimated that 10-15% of the dye is lost in the effluent during the dyeing process [2].

The waste water discharged from industries is either used for irrigation purposes or it runs off into natural sources of water. Thus waste water from textile industries creates a big pollution problem due to the dye content presence in it. The inadequacy in dying process has resulted in 10-15% of unused dye stuff entering the waste water directly [3].

This wastewater consist high contents of other products besides dye compounds such as dispersants, acids, bases, salts, detergents and oxidants. Even a small amount of dye present in pure water is highly visible and undesirable. Therefore, discharges from textile industries are usually high in colour content, biological oxygen demand, chemical oxygen demand and suspended solids [4]. The direct discharge of textile wastewater into the water stream is certainly impermissible and every one of us is being exposed to contamination from the past and present industrial practices. In addition these textile dyes reduce light penetration, affect the photosynthetic activity in aquatic life and it may also be toxic and carcinogenic to living organisms. Thus, it is necessary to develop an effective and efficient method to remove the colour from waste water before being discharged into natural water stream [5]. Dyes used in the textile industries are classified into three classes (a) anionic (b) cationic (c) non-ionic. Basic and reactive dyes are extensively used in the textile industry because of their favourable characteristics of bright colour, being easily water soluble, cheaper to produce and easier to apply to fabric [6].

History of dyes

The first human made(synthetic) organic dye, mauveine, was discovered by william henry perkin in 1856. Many thousands of synthetic dyes have since been prepared. Synthetic dyes quickly replaced the traditional natural dyes. They cost less, they offered a vast range of new colours, and they impaired better properties upon the dyed materials. Archaeological evidence shows that, particularly in India and the middle east, Dyeing has been carried out for over 5000 years. The dyes were obtained from animals, vegetable or mineral origin, with no or very little processing. By far the greatest secure of dyes has been from the plant kingdom, notably roots, berries, bark, leaves and wood ,but only a few have ever been used on a commercial scale [7]. A dye can generally be described as a coloured substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution and may require a mordant to improve the fastness of dyes on the fiber. Both dyes and pigments appear to be coloured because they absorb some wavelengths of light preferentially. In contrast with a dye, a pigment generally is insoluble, and has no affinity for the substrate. Some dyes can be precipitated with an inert salt to produce a lake pigment.

Classification of dyes

All molecules absorb electromagnetic radiation, but differ in the specific wavelengths absorbed. Some molecules have the ability to absorb light in the visible spectrum (400-800nm) and as a result, they are themselves coloured. The dyes are molecules with delocalized electron systems with conjugated double bonds that contain two groups, the chromophore and the auxochrome. The chromophore is a group of atoms, which controls the colour of the dye, and it is usually an electron-withdrawing group. The most important chromopphores are-c=cc--,--cc=n-,-c=o-,=n=n-,-no2 and –no groups. The auxochrome is an electron donating substituent that can intensify the colour of the chromophore by altering the overall energy of the electron system and provides solubility and adherence of the dye to the fiber. The most important auxochromes are-NH2,-NR2,_NHR,-COOH,-SO3H,-OH and-OCH3 groups. Based on the chemical structure or chromophore,20-30 different dye groups can be identified.Azo (monoazo, diazo, triazo, polyazo),anthraquinone, phthalocyanine and triarylmethane dyes are quantitatively the most important chromophores. Other groups are diartlmethane, indigoid, azine, oxazine, thiazine, xanthenes, nitro, nitroso, methine, thiazole, indamine, indophenols, lactone, aminoketone and hydroxyketone dyes of undetermined structure (stilbene and sulphur dyes) [8]. Most of the commercial dyes are classified in terms of colour, structure or method of application in the colour index (C.I.), which is edited every three months since 1924 by the “society of dyes and colourists” and the “American association of textile chemists and colourists”. The last edition of the colour index lists about 13000 different dyes. Each dye is assigned to na C.I.generic name determined by its application and colour [9].

Acid dyes: Acid dyes are highly water soluble due to the presence of sulphonic acid groups. The largest class of dyes in the color index is referred to as acid dyes(-2300 different acid dyes listed,-40% of them are in current production).Acid dyes are anionic compounds that are mainly used for dyeing nitrogen-containing fabrics like wool, polyamide, silk, and modified acryl. They bind to the cationic NH4+ ions of those fibers. Most acid dyes are azo(yellow to red or a broader range colours in case of metal complex azo dyes), anthroquinone or triarylmethane (blue and green) compounds. The adjective 'acid' refers to the pH in acid dye rather than to the presence of acid groups (sulphonate, carboxyl) in the molecular structure of these dyes [10].

Reactive dyes: Reactive dyes are dyes with reactive groups that form covalent bonds with OH-,NH-, or SH-groups in fibers (cotton, wool, silk ,nylon).The reactive group is often a heterocyclic aromatic ring substituted with chloride or fluoride, e.g.dichlorotriazine. Another common reactive group is vinyl sulphone (as in reactive orange 7).The use of reactive dyes has increased ever since their introduction in 1956, especially in industrialized countries. In the colour index, the reactive dyes from the second largest dye class with respect to the amount of active entries, about 600 of the 1050 different reactive dyes listed are in current production. During dying with reactive dyes, hydrolysis (i.e.inactivation) of the reactive groups is an undesired side reaction that lowers the degree of fixation. Inspite of the addition of high quantities of salt and ureum (up to respectively 60 and 200 g/l) to raise the degree of fixation, it is estimated that 10 to 50% will not react with the fabric and remain hydrolysed in the water phase. The problem of coloured effluents is therefore mainly identified with the use of reactive dyes. Most (-80%) reactive dyes are azo or metal complex azo compounds but also anthraquinone and phthalocyanine reactive dyes are applied, especially for green and blue [11].

Direct dyes: Their flat shape and length enables them to bind along-side cellulose fibers and maximize the van der walks, dipole and hydrogen bonds. Only 30% of the 1600 structures are still in production due to their lack of fastness during washing. The most common structures are almost always sulphonated azo dyes [12].

Basic Dyes: Basic dyes work very well on acrylics due to the strong ionic interaction between dye functional groups such as –NR3+ or=NR2+ and the negative charges in the copolymer. The most common structures are azo, diarylmethane, triarylmethane and anthraquinone.

Mordant Dyes: Mo0rdants are usually metals salts such as sodium or potassium dichromate. They act as "fixing agent" to improve the colour fastness. They are used with wool, leather, silk and modified cellulose fibers. The most common structures are azo, oxazine or triarylmethane [13].

Disperse Dyes: Disperse dyes are non-ionic structure, with polar functionality like –NO2 and –CN that improve water solubility, van der wals forces, dipole forces and the color. They are usually used with polyester. The most common structures are azo, nitro, anthraquinones or metal complex azo.

Pigment Dyes: These insoluble, non-ionic compounds or salts, representing 25% of all commercial dye names, retain their crystaline or particulate structure throughout their application. The most common structures are azo or metal complex phthalocyanines.

Vat Dyes: Vat dyes are insoluble in water , but may become solubilised by alkali reduction(sodium dithionite in the presence of sodium hydroxide).The produced leuco form is absorbed by the cellulose(van der waals forces)) and can be oxidized back, usually with hydrogen peroxide , to its insoluble form. The most common structures are anthraquinones or indigoids [14].

Ingrain Dyes: The term 'ingrain' is applicable to all dyes formed in situ ,in or on the substrate by the development, or coupling of one or more intermediate compounds and a diazotized aromatic amine. In the colour index the sub-section designated ingrain is limited to tetra-azaporphin derivatives or precursors.

Sulphur Dyes: Sulphur dyes are complex polymeric aromatics with heterocyclic SS-containing rings representing about 15% of the global dye production. Dyeing with sulphur dyes (mainly on cellulose fibers) involves reduction and oxidation processes, comparable to vat dyeing.

Solvent Dyes: Solvent dyes are non-ionic dyes that are used for dyeing substrates in which they can dissolve as plastics, varnish, ink and waxes. They are not often used for textile processing. The most common structures are diazo compounds that undergo some molecular rearrangement, triarylmethane, anthraquinone and phthalocyanine [15].

Food Dyes: Food dyes are other class which describes the role of dyes, rather than their mode of use. Because food dyes are classed as food additives, they are manufactured to a higher standard than some industrial dyes. Food dyes can be direct, mordant and vat dyes and their use is strictly controlled by legistlation .Many are azoic dyes, although anthraquinone and triphenylmethane compounds are used for colours such as green and blue. Some naturally occurring dyes are also used.

Fluorescent brighteners: Fluorescent brighteners (or bluing agents) mask the yellowish tint of natural fibers by absorbing ultraviolet light and weakly emitting visible blue. They are not dyes in the usual sense because they lack intense colour. Based on chemical structure, several different classes of fluorescent brightners are discerned: stilbene derivatives, coumarin derivatives, pyrazolines, 1,2-ethane derivatives, naphthalimides and aromatics or heterocyclic ring structures. Many fluorescent brighteners contain triazinyl units and water solubilising groups [16].

Other Dye classes: Food dyes are not used as textile dyes. Natural dyes use in textile processing operations is very limited. Fluorescent brighteners mask the yellowish tint of natural fibers by absorbing ultraviolet light and weekly emitting blue light. Not listed in a separate class in the color index, many metal complex dyes can be found (generally chromium, copper, cobalt or nickel).The metal complex dyes are generally azo compounds.

Application of Dyes

- Textile industry
- Paper industry
- Food industry
- Cosmetics industry
- Pharmaceutical industry
- Polymer industry
- Leather industry

Sources of Dyes Pollution

Dyes are a kind of organic compounds which can bring bright and firm colour to other substances. Synthetic dyes usually have a complex aromatic molecular structure which possibly comes from coal tar based hydrocarbons such as benzene, naphthalene, anthracene, toluene, xylene, etc. The complex aromatic molecular structures of dyes make them more stable and more difficult to biodegrade. Today there are more than 10000 dyes are available commercially. Synthetic dyes have been increasingly based in the textile, leather, paper, rubber, plastics, cosmetics, pharmaceuticals and food industries. The extensive use of dyes often poses pollution problems in the form of coloured waste w3ater discharged in to environmental water bodies. For some dyes, the dye concentration of less than 1 mg/l in receiving water bodies is highly visible, so that even small quantities of dyes can colour large water

bodies. This not only affects aesthetic merit but also inhibits sunlight penetration and reduces photosynthetic action. In addition, some dyes or their metabolites are either toxic or mutagenic and carcinogenic. Water pollutants are categorized as pointy source or nonpoint source, the former being identified as all dry weather pollutants that enter watercourses through pipes or channels. Storm drainage, even though the weather may enter watercourses by way of pipes or channels, is considered nonpoint source pollution. Other nonpoint source pollution comes from agricultural runoff, construction sites and other land disturbances [17].

Treatment Methods for Dye Removal

A wide range of dye removal techniques have been studied and developed remove dyes from the wastewater before discharging to the environment. These techniques include adsorption, coagulation- flocculation, reverse osmosis, oxidation, membrane filtration and microbial degradation. Adsorption and biodegradation are most effective process for removal of dyes from wastewater. Activated carbon is popular and effective dye sorbent, but its relatively high price, high operating costs and problems with regeneration hamper its large scale application. Therefore, there is a growing need in finding low cost, renewable, locally available materials as sorbent for the removal of dye. Some eco-friendly low cost materials (treated and untreated forms) have been used as sorbent for dye removal from wastewater. Aerobic, anaerobic and combined anaerobic –aerobic bioreactors are used for the degradation of dyes from wastewater.

Low cost adsorbents are becoming the focus of many researchers. These adsorbents could be produced from many raw materials such as agricultural by products/wastes and industrial waste products. The conventional treatment methods for dye effluents, such as oxidation, coagulation and flocculation, photo catalytic destruction, ion exchange and nano membrane filtration. But these are complicated and costly, in particular as some methods require additional chemicals or may produce toxic by products. At present research is focussed on the bio sorbent for the dye removal mainly by bacteria, fungi, micro and macro algae, agricultural by products and other polysaccharides materials [18]. With the search of a new dimension of treatment methods, the biological techniques of dye decolourisation are cheaper and easier to operate when compared to the conventional treatments like physical and chemical methods. Removal of dye from aqueous solution using bio sorbents is also a nonconventional technology and it mainly takes place on the biomass surface and the binding sites at the surface is activated and thus increase the effective approach of enhancing the biosorption capacity. The main advantages of sorption treatment for the control of water pollution are less investment in terms of initial development cost, simple design, easy operations, no generation of toxic substances and easy and safe recovery of the adsorbent. A large variety of adsorbent materials have been used to adsorb the dye molecules such as fruit peels ,wood, banana pith, maize cobs, barley husk and bagasse pith, zeolites, activated clays, activated carbons, palm kernel fiber, red mud, bottom ash [19]**.**

Ion exchange: Standard ion exchange systems have not been widely used for treatment of dye-containing effluents, mainly due to the opinion that ion exchangers cannot accommodate a wide range of dyes and dyeing conditions and that their performance was greatly affected by the presence of additives in the wastewater. In this technique wastewater is passed over the ion exchanger resin until all available exchange sites are saturated. Both anionic and cationic dyes are efficiently removed by this method .A disadvantage of this method is the high cost of organic solvents to regenerate the ion-exchange [20].Table 1 shows the wastewater treatment and its operation.

Coagulation/Flocculation: This method is often applied in the treatment of different types o wastewaters and it is used to enhance the degree of removal of total suspended solids(TSS),biochemical oxygen demand(BOD),chemical oxygen de4mand(COD) and colour. The first step, coagulation, consists in the addition of a coagulant to the wastewater and mixing. This coagulant destabilizes the colloidal particles that exist in the suspension, allowing particle agglomeration. Flocculation is the physical process of bringing the destabilized particle in contact to form larger flocs that can be more easily removed from the solution. This is usually achieved by a slow mixing step. The most commonly used inorganic coagulants/flocculants are: trivalent salts of iron and aluminium, ferrous sulphate

and calcium hydroxide/lime. These are often used with various coagulants aids, such as synthetic polyelectrolytes, fly ash and clay. Inorganic compounds are however generally not very suitable to remove highly soluble dyes from solution unless large quantities are dosed. They major disadvantages of t6he use of this process is the amount of useless and even toxic sludge that needs to be correctly disposed and the possibility of a secondary pollution problem. Recently some organic polymers have been developed with good dye coagulant properties and a relatively low sludge production.

Photochemical: Process like UV/H2O2,UV/TiO2,UV/Fent5ons reagent , UV/O3 and others are photochemical methods based on the formation of free radicals due to UV irradiation. Degradation is caused by the production of high concentrations of hydroxyl radicals and the dye molecule is degraded to CO2 andH2O. The rate of dye removal is influenced by the intensity of the UV radiation, pH, dye structure and the dye bath composition. When H2O2 is used as oxidizing agent, the UV light activates the decomposition of H2O2 into two hydroxyl radicals. This method does not produce sludge and greatly reduces foul odours. There is also the possibio9lity of effectively using sunlight or near UV light for irradiation, which would result in considerable economic savings, especially for large-scale operations. Faster, cheaper and more effective photocatalytic process are based on catalysis by solid semiconductor materials, mostly TiO2 particles. With TiO”2 catalyzed UV treatment a wide range of dyes can be mineralised. The photodegradation of dyes by this method depends considerably on the chemical structure of the dye [21].

Electrolysis: Electrolysis is based on applying an electric current through to the wastewater by using electrodes. Organic compounds like dyes react through a combination of electrochemical oxidation, electrochemical reduction, electro coagulation and electro flotation reactions. For instance, when iron is the sacrificial anode. Fe(II)-ions are released to the bulk solution and acid dyes are sorbed on the precipitated Fe(OH)2.Moreover Fe(II) can reduce azo dyes to arylamines . Moreover, water can also be oxidized resulting in the formation of O2 and O3 and if chloride is present, there is also formation of Cl2 and oxychloroide anions. In the cathode occurs reduction of water to H2 and OH-. In order to improve the performance of the system different materials have been tested in the electrodes like carbon-fiber, Ti/Pt and aluminium. The main disadvantage of these types of methods is the cost, both initial capital costs, energy and of electrode replacement. The formation of unwanted breakdown products and foam are also drawbacks of this method. The main advantages are compact size of equipment, simplicity in operation, fast rate of pollutant removal and decrease amount of sludge generated. The method is efficient for colour, BOD, CODF, TOC, TDS, TSS and heavy metals removal.

Adsorption: Adsorption has been found to be cheap, effective and easy method for the treatment of dyes among the above physic-chemical treatment processes. Adsorption operations exploit the ability of certain solids preferentially to concentrate specific substances from solution onto their surfaces. Adsorption on a carbon bed has been used in waste water treatment for many years. The process is based on intimate contact between the waste water and carbon bed, on which the waste materials are adsorbed. The clear liquid is then separated from the adsorbent. In the case of the packed bed or expanded bed adsorption system, the water is passed through the adsorbent. Efficient and economical regeneration of the bed is a pre requisite for the application of the process of waste water technology [22].

Biological Techniques: Biodegradation is the process by which organic substances are broken down by other living organism. The term is often used in relation to ecology, waste management, environmental remediation (bioremediation) and to plastic materials, due to their long life span. Organic material can be degraded aerobically, with oxygen or anaerobically without oxygen. A term related to biodegradation is bio mineralisation in which organic matter is converted in to minerals. Biodegradable matter is generally organic material such as plant and animal matter and other substance originating from living organisms. Biological treatment processes are very useful and remove all types of dissolved substances which are hazardous to the environment. The biological treatment of wastewater in the wastewater treatment plant often accomplished by means of the application of conventional

activated sludge systems. In recent years, new technologies are being developed to improve this system. The use of aerobic granular sludge is one of them. Biological treatment is divided into two categories viz. Anaerobic and aerobic.

Biosorption

Sorption is a term used for both absorption and adsorption. Absorption is the incorporation of a substance in one state into another different state (i.e., liquid being absorbed by a solid or a gas). Adsorption is the physical adherence or bonding of ions and molecules on to the surface of the solid material. In the adsorption the materials accumulated at the interface is the adsorbate and the solid surface is the adsorbent. Biosorption is a subcategory of adsorption where the sorbent is a biological matrix. It is a process of rapid and reversible binding of ions from aqueous solution with functional groups present on the surface of biomass. According to gad it is the removal or binding of soluble pr insoluble substance from aqueous solution by biological materials. This process is efficient, selective and independent of cellular metabolism. It can be performed in a wide range of pH and temperature using optimum particle size of bio sorbent. Biological materials are often inexpensive a=nd can be obtained from agricultural waste [23].

History of biosorption: Although the ability of living microorganism to take up dyes from aqueous solution was investigated as early as 18th and 19th centuries, it is only during the last three decades that living microorganism have been used as adsorbents for removal and recovery of materials from aqueous solution. The earliest technological application of biosorption involved sewage and waste treatment. It was investigated for use in renovating waste water generated by the chemical industry. The first patent for a biosorption apparatus used for biological apparatus used for biological treatment of waste water was registered by Ames crosta M ill and company ltd. In1973. Researchers in life sciences primarily focused on the toxicological effects and accumulation of dyes in micro organisms, while environmental scientists and engineers used this capacity of micro organisms and a means of monitoring dye pollution as well as for removal or recovery of dyes from waste waters. Some review papers have reported that the first quantitative study on dye biosorption was done. The practical use of biosorption technology for monitoring trace dyes in the environment. Till date biosorption technique is considered as an ideal alternative for removal of dye from an effluent.

Biosorbent: For removal of a desired pollutant , a variety a biomatertials have been used as the biosorbent and include all types of microbial, plant and biomass and their derivative products. In recent years more attention has been given towards agriculture waste, industrial waste and polysaccharides biomaterials. Microorganism like bacteria, cyanobacterial algae, yeast, fungi, and even lichens are also used for removal and recovery of dyes due to t5heir good performance , low cost and availability in large quantity. All biological materials have a great affinity for metal ions because of presence of abundant chelating functional groups. Out of these biomaterials chitosan has widely used to treat a large number of aquatic pollutants due to its high content of amino and hydroxyl functional groups. Usually biosorbents are prepared from the naturally abundant waste biomass by inactivation with acid or base before the final drying. Some biomasses are used with synthetic polymer matrix or inorganic supporting material such as silica. Nowadays simple cutting or grinding of dry biomass provides a stable biosorbent with desired particle size. In general biosorbent being a dead biomass exhibits more advantages in comparison with the use of living micro organisms as biosorbent, because dead cells can be easily stored and used for a longer time. Dead biomass is not the subject to metal toxicity limitations, nutrient supply is not required And dye loaded biosorbent can be easily desorbed and reused [24].

Mechanism of biosorption: The process of biosorption involves a solid phase (sorbent/biosorbents/adsorbent biological materials) and liquid phase (adsorbate.dye solution). Due to the higher affinity of the adsorbent for the adsorbate species, the latter is attracted and bound thereby different mechanisms to the adsorbent. The process continues till equilibrium is established between the amount of solid bound adsorbate species and its portion

remaining in the solution. The degree of adsorbent affinity for the adsortbate determines its distribution between the solid and liquid phase. Special surface properties of bacteria, fungi, yeast and algae enable them to adsorb different kinds of pollutants from solution. During biosorption process a number of metabolism independent processes such as physical adsorption, chemical adsorption, electrostatic interactions may occur essentially in the cell wall rather than oxidation through anaerobic metabolism. The mechanism of binding depends upon the type of biomass, chemical nature of the pollutant and the environmental conditions such as pH, temperature and ionic strength. The probable mechanisms for adsorption are described as follows

Physical adsorption: It is also known as physisorption. There is no exchange of electrons and there occurs intermolecular attraction between favourable energy sites which is independent of electronic properties of involved molecules. In this mechanism van der waals interactions exist between the adsorbate and adsorbent. These interactions have a long range but are weak and release energy (Enthalpy of condensation), when a particle is physisorbed. Such small energies can be adsorbed as vibrations of the insufficient to cause breaking of bond. Sob a physisorbed molecules retains its identity, although it might be distorted by the presence of the surface.

Chemical adsorption: Chemical adsorption (chemisorption) involves an exchange of electrons between specific surface sites and solute molecules and as a result a chemical bond is formed. In chemical adsorption, the molecules or atoms stick to the surface by forming a chemical bond usually co-valent bond and tend to find sites that maximize their co ordination number with the substrate. The enthalpy of chemisorptions is much greater than that for physisorption. Generally only a single molecular layer can be adsorbed. Chemical adsorption may be rapid or slow and may occur above and below the critical temperature of the adsorbate. Chemisorption is further divided into ion exchange, complexation and chelation. Ion exchange is a reversible chemical reaction where an ion of a solution is exchanged for a similarly charged ion attached to an immobile solid particle. These reactions are stoichiometric and reversible. Chilation is the firm binding of a dye with an organic molecule (ligand) to form a ring structure while complexation is any combination of cation with anions containing free pairs of electrons [25].

Biochemistry of biosorption: Biosorption is the ability of biological material to accumulate dyes from waste water through metabolic processes or by physico chemical uptake. Inbiological materials the capacity of metal binding varies due to effect of several factors on specific bio sorbents. The composition of the cell wall is of great importance to the biosorption process. The cell wall of biomass is composed mainly of polysaccharides, proteins and lipids having a number of functional groups like hydroxyl, carboxyl, amino, ester, sulphydryl, carboxyl terminal, carboxyl internal which play a key role in the biosorption of cautions from aqueous solution. During biosorption process protons and/ or light metal cations which are naturally bound to functional groups located on the surface of biomass are exchanged with dye present in aqueous solution. The process of biosorption involves different groups that function as binding sites. Oxygen atom from carboxyl group of alginic acid, sulphur atom from thiol and sulfonate groups of amino acid, sulphate, polysaccharides, fucoidan while nitrogen atom from amine and amide groups of amino acids, peptidoglycan, act as main binding sites which are present in cell wall matrix of seaweeds.

Factors influencing biosorption: For the industrial application of biosoprption technology it is very important to investigate the removal efficiency of a given biosorbent for the target pollutant. Pollutant uptake can involve different types of biosorption processes that will be affected by various physical and chemical factors and these factors will determine the individual of co operative effects of various factors on biosorption. In batch biosorption process used for removing adsorptive pollutants like metals or dyes , the important factors that influence the process include pH, temperature, initial pollutant concentration, biosorbent dosage, biosorbent size, agitation speed and concentration of other pollutants. Effect of these factors on removal of dyes is summarized in Table 2 below [26].

CONCLUSION

Biosorption processes have attached a great importance from an environmental point of view as they can be used to remove toxic compounds from industrial wastewaters. Many industries use dyes to colour their final products and

their discharge into natural waters causes severe problems because they are toxic to aquatic life and damage the aesthetic nature of the environment. Moreover, these effluents are rather difficult to treat by conventional biological and physical-chemical techniques due to the complex structure of the dyes. Therefore, adsorption processes provide an attractive alternative for the treatment of coloured waters. Activated carbon is the most popular and widely used adsorbent, but there are certain problems with its use since it is expensive and its regeneration is difficult. For this reason, interest has been recently focused on low-cost materials, ranging from waste products from other industries to naturally abundant biomass: sewage sludge and peanut shell, silk cotton hull, coconut tree saw dust and coir pith, moss, banana pith and water hyacinth roots, parthenium plant, bacteria and fungi. In particular, marine algae are very promising materials to be used as biosorbents in wastewater treatment because they represent a cheap source of biosorbent, as they are readily available in large quantities, and it has been shown that they display a high metal binding capacity, mainly due to carboxylic and sulfonate groups from the algal polysaccharides.

REFERENCES

1. Geise GM, Lee HS, Miller DJ, Freeman D, Mcgrath JE, Paul DR. Water purification by membranes: The role of polymer science. J Pol Sci 2010;48:1685-1718.
2. Ahmad AL, Harris WA, Syafiie, Ooi BS. Removal of dye from wastewater of textile industry using membranes. J Tek. 2012:36;31-44.
3. Tomczak ET, Kamiński W, Tosik P. Adsorption dynamics studies of azo dyes removal by biosorbent. Desalin Water Treat. 2014;55(10): 2669-2674.
4. Tomczak ET, Kamiński W, Szczerkowska D. Fractional derivatives for description of sorption kinetics in the plant sorbent-metal ions system. Ecol Chem Eng 2013;20(3):499- 506.
5. Crini G. Non-conventional low-cost adsorbents for dye removal: A review. Biores Technol. 2006;97:1061-1085.
6. Vadivelan V, Kumar KV. Equilibrium, kinetics, mechanism, and process design for the sorption of methylene blue onto rice husk. J Colloid Interface Sci. 2005;286:90-100.
7. Wang Y, Gao BY, Yue WW, Yue QY. Adsorption kinetics of nitrate from aqueous solutions onto modified wheat residua. Colloids Surf A. Physicochem Eng Aspects. 2007;308:1-5
8. Tan IAW, Ahmad AL, Hameed BH. Adsorption of basic dye on high-surface-area activated carbon prepared from coconut husk: Equilibrium, kinetic and thermodynamic studies. J Hazard Mater. 2008;154:337-346.
9. Sheng PX, Ting YP, Chen JP, Hong L. Sorption of lead, copper, cadmium, zinc, and nickel by Marine algalbiomass: characterization of biosorptive capacity and investigation of mechanisms. J Colloid Interf Sci.2004;275:131-141.
10. Rajfur M. Algae-heavy metals biosorbent. Ecol Chem Eng S. 2013;20:23-40.
11. Robinson T, Chandran B, Nigam P. Removal of dyes from an artificial textile dye effluent by two agricultural waste residues, corncob and barley husk. Environ Internat. 2002;28:29-33.
12. Nada AAM, Mahdy A, El-Gendy AA. Spectroscopy and chemical studies of cation exchangers prepared from corn cobs. Bio Resources. 2009;4(3):1017-1031.
13. Fonseca-Correa F, Giraldo L, Moreno-Pirajan JC. Trivalent chromium removal from aqueous solution with physically and chemically modified corncob waste. J Analyt Appl Pyrolysis. 2013;101:132-141.
14. Shen J, Duvnjak Z. Adsorption kinetics of cupric and cadmium ions on corncob particles. Process Biochem.2005;40:3446-3454.
15. Leyva-Ramos R, Bernal-Jacome LA, Acosta-Rodriguez I. Adsorption of cadmium(II) from aqueous solution on natural and oxidized corncob. Separation Purif Technol. 2005;45:41-49.
16. Nasiruddin Khan M, Farooq Wahab M. Characterization of chemically modified corncobs and its application in the removal of metal ions from aqueous solution. J Hazard Mater. 2007;6:237-244.
17. Zhang C, Geng Z, Cai M, Zhang J, Liu X, Xin H, et al. Microstructure regulation of super activated carbon from biomass source corncob with enhanced hydrogen uptake. Internat J Hydrogen En. 2013;38:9243-9250.
18. El-Hendawy AA, Samra SE, Girgis BS. Adsorption characteristics of activated carbons obtained from corncobs. Colloids Surf A: Physicochem Eng Aspects. 2001;180:209-221.

19. Tseng RL, Tseng SK. Pore structure and adsorption performance of the KOH-activated carbons prepared from corncob. Desalination. 2011;280:1-13.
20. Sych NV, Trofymenko SI, Poddubnaya OI, Tsyba MM, Sapsay VI, Klymchuk DO, et al.Porous structure and surface chemistry of phosphoric acid activated carbon frocorncob.Appl Surf Sci. 2012;261:75-82.
21. Yong S, Webley PA. Preparation of activated carbons from corncob with large specific surface area by a variety of chemical activators and their application in gas storage. Chem Eng J. 2010;162:883-892.
22. Aworn A, Thiravetyan P, Nakbanpote W. Preparation of CO2 activated carbon from corncob formonoethylene glycol adsorption. Colloids Surf A: Physicochem Eng Aspects.2009;333:19-25.
23. Wu FCh, Wu PH, Tseng RL, Juang RS. Preparation of novel activated carbons from H2SO4-pretreatedcorncob hulls with KOH activation for quick adsorption of dye and 4-chlorophenol. J Environ Manage.2011;92:708-713.
24. Preethi S, Sivasamy A, Sivanesan S, Ramamurthi V, Swaminathan G. Removal of safranian basic dye fromaqueos solutions by adsorptions onto corncob activated carbon. Eng Chem Res. 2006;45:7627-7632.
25. Reddad Z, Gerente C, Andres Y, Le Cloirec P. Adsorption of several metal ions onto a low-cost biosorbent:Kinetic and equilibrium studies. Environ Sci Technol. 2002;36:2067-2073.
26. Sud D, Mahajan G, Kaur M.P. Agricultural waste material as potential adsorbent for Sequester in heavy metal ions from aqueous solutions - A review. Biores Technol. 2008;99:6017-6027.

Table 1: Wastewater Treatment and its operation

Treatment	Operations
Primary	Screening, Sedimentation, Equalisation, Neutralisation, Mechanical flocculation and chemical coagulation
Secondary	Aerated lagoon, Trickling filtration, Activated sludge process, Oxidation ditch and pond, Anaerobic digestion
Tertiary	Oxidation technique, Electrochemical process, Ion exchange method, Photocatalytic degradation, Adsorption, Thermal evaporation, Membrane technologies, Electrolytic precipitation and foam fractionation.

Table 2: Effect of physical factors on biosorptive removal of dyes

S. no	Process factor	Effect on biosorption
1	pH	Enhances removal of cationic or basic dyes but reduces that of anionic or acidic dyes
2	Initial pollutant concentration	Increases the quantity of biosorbed pollutant per unit weight of biosorbent but decreases its removal efficiency
3	Biosorbent dose	Decreases the quantity of biosorbed pollutant per unit weight of biosorbent but increases its removal efficiency
4	Biosorbent size	It is favourable for batch process due to higher surface area of the biosorbent but not for column process due to its low mechanical strength and clogging of the column
5	Agitation speed	Enhances biosorptive removal rate of adsorptive pollutant by minimizing its mass transfer resistance, but may damage physical structure of biosorbent
6	Other pollutant concentration	Reduces biosorptive removal of the target pollutant
7	Temperature	Enhances biosorptive removal of adsorbate by increasing surface activity of the adsorbate but damages physical structure of biosorbent.

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RESEARCH ARTICLE

Environmental Benign Synthesis and Spectroscopic Study of Metal Complexes of Cu(II), Co(II), Ni(II), Fe(II) using Pyrazoline Derivatives

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ABSTRACT

Synthesis of a series of metal complexes of Cu(II), Co(II), Ni(II), Fe (II) by using new bidented pyrozoline ligands namely (L2) 3-(4-bromophenyl)-5-(4-chlorophenyl)4, 5-dihydro-1H-pyrazole, (L3) 3-(4-bromophenyl)-5-(4-methylphenyl)4, 5-dihydro-1H-pyrazole, (L4) 3-(4-methylphenyl)-5-(4-chlorophenyl)4, 5-dihydro-1H-pyrazole, (L5) 3-(4-bromoophenyl)-5-(4-phenylethenyl) 4, 5-dihydro-1H-pyrazole and (L6) 3-(4-chloroophenyl)-5-(4-phenylethenyl)4,5-dihydro-1H-pyrazole. This synthesized Ligands structures were confirmed by NMR and IR spectral techniques and metal complexes structures were confirmed by NMR and TGA spectral studies.

Keywords: Ligands, Metal Complexes, Acetophenone, Aldehyde, Pyrazoline, NMR, IR, TGA

INTRODUCTION

As coordination compounds encloses so many fields such as medicinal chemistry, pharmaceuticals, diagnostics, ceramics, petrochemicals, dye industries, metal extraction, plastic industry, catalysis, organic photovoltaic materials. Pyrazoline derivatives and their metal complexes are associated with pharmacological and much more applications in industries. The substituted 2-pyrazolines used as activators for polymerization[1], dyes for wool, nylon [2], as electro photographic conductors [3] and as wavelength shifters in liquid and polymer scintillation [4].Different pyrazoline based metal complexes are used as anticancer agent, medical diagnostic probes [5,6]. However this structural and biological properties of pyrazoline derivatives and their metal complexes indicates that extensive work has been carried out on metal complexes of pyrazoline derivatives. So that sequentially chalcones , pyrazoline and metal complexes were synthesized and spectrally studied with NMR, IR and TGA.

METHODS AND MATERIALS

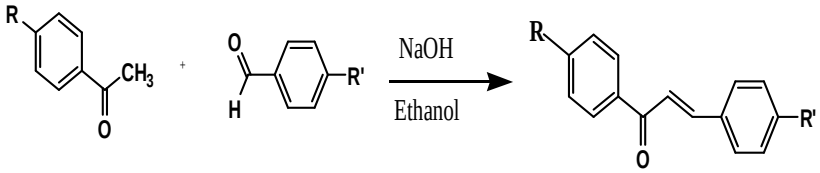
The A.R grade chemicals were used which obtained from known chemical companies like Lancaster and Sigma–Aldrich. The purification of ethanol solvent was done by standard distillation method[7]. Hydrated Metal salts such as sulphates of copper and iron, chlorides of cobalt and nickel were used for synthesis of metal complexes.

Experimental Procedure

The experimental procedure includes : a) synthesis of α,β-unsaturated ketones as precursor to preparation of ligand b) Synthesis of Pyrazoline Derivatives as Ligand c) Synthesis of metal complexes of Cu(II), Co(II), Ni(II), Fe(II) using pyrazoline derivatives .

Synthesis of α, β-unsaturated ketones as precursor to preparation of ligand

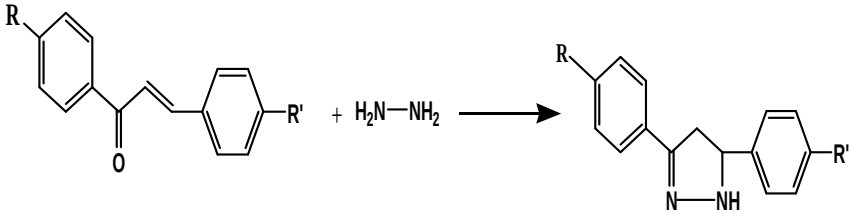
10 millimoles substituted acetophenone and 10 millimoles of substituted benzaldehyde or cinnamaldehyde (equimolar quantities) in 20 ml ethanol was taken into RB flask stirred on magnetic stirrer upto dissolution. 20 millimoles of NaOH was dissolved into minimum amount of water and added into RB in dropwise manner. Solid precipitate was observed stirred for 24 hours . To increase yield of product, reaction was worked up. The precipitate was filtered and recrystallized from ethanol. The recrystallized precipitate (α, β-unsaturated ketones) was dried used as precursor to preparation of ligand.



Acetophenone Benzaldehyde α, β-unsaturatedketones

Synthesis of pyrazoline derivatives as ligand

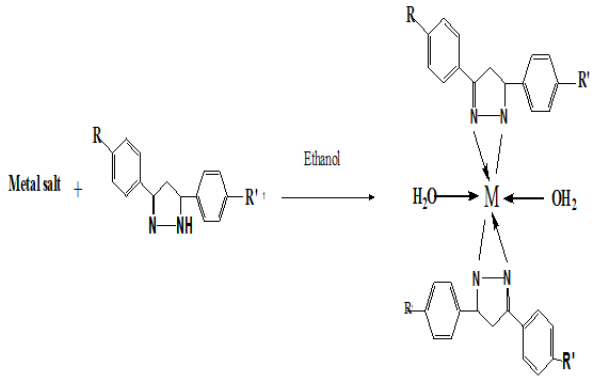
4 millimoles of α,β-unsaturated ketones and 28 millimoles of hydrazine hydrate in 20 ml ethanol was taken into round bottom flask was refluxed for 6-8 hours. The mixture was kept overnight. Concentration of ethanol was decreased by evaporation. Mixture was cooled into ice bath. Product was separated by simple filtration method and recrystallizes from pure ethanol and dried.



α, β-unsaturated ketones Hydrazine Hydrate Ligand

Synthesis of metal complexes of Cu(II), Co(II), Ni(II), Fe(II) using pyrazoline derivatives

2 millimoles of ligand was dissolved into 10 ml ethanol taken in round bottom flask on magnetic stirrer. After complete dissolution of ligand 1 millimoles of metal salt was added and the solution was stirred upto overnight. The colourful precipitate was obtained. The precipitate was filtered and washed with excess of ethanol and dried. The other metal complexes were prepared by similar procedure.



RESULT AND DISCUSSION

All the metal complexes and pyrazoline derivatives were stable at room temperature. The structures of pyrazoline derivatives were confirmed by NMR and IR Spectra

NMR and IR spectral analysis for Ligands

NMR spectra for ligands

A pyrazoline ring is identified by characteristic spectral features[8] in it's ^{1}HNMR spectrum. The three protons in the pyrazoline ring H1, H2, H3, were seen as a doublet of doublets at δ 4.91 to 2.39 ppm respectively, due to the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring. A singlet in the range of δ 4.68-5.62 ppm corresponds to -NH of pyrazoline ring. Aliphatic protons were observed in expected regions. Methyl group was observed at 2.68-2.87ppm. All such types of peaks were observed according to the reported literature values [9].

IR spectra for ligand

The IR spectra of ligands showed the absorption bands in the region of 1670-1680 cm^{-1} due to -C=N- of pyrazoline. The absorption band with sharp peaks at 3300-3500 cm^{-1} is due to -NH stretching. Whenever Beside these bands, 700-800 cm^{-1} due to C-Cl stretching and 600-700 cm^{-1} due to C-Br andappears whenever present in the respective compound. And pyrazoline shows aromatic C-H bonding in lower region than aliphatic in bending region. These observations shows strong agreement for pyrazoline structure [10,11].

IR and TGA spectral analysis for Metal complexes

IR Spectra

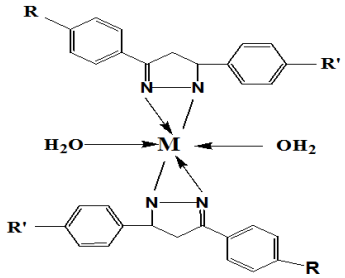
The ligand shows sharp pointed peaks in region 3330-3340 due to -NH bonds was completely disappeared in remarkable changes of new broad bands around 3330-3480cm^{-1} assigned to vibration modes of -OH of coordinated water molecules. In addition to that metal oxygen (M-O) bond is observed in range 430-485cm^{-1}. This information clearly indicates that water molecule is coordinated with metal complexes. The ligands shows (C=N) in the region 1670-1680 cm^{-1} of pyrazoline ring show remarkable changes in position of (C=N) in the region of 1580-1600 cm^{-1}incase of metal complexes. This shifting of position of (C=N) is due to (M-N) attachment. The lower frequency regions of IR spectra of all complexes recorded weak bands around 520-560 cm-1 that are attributed to M-N bonds [12,13]. The data obtained from FT-IR spectra investigated the bi dentate behaviour of the ligand through two nitrogen atoms of pyrazoline and metal complexes formation.

TGA spectral analysis for Metal complexes

TGA analysis is very important which carried out to confirm presence of water molecule in these complexes as well as to know their decomposition pattern. This analysis correlate the information obtained from the IR spectral studies. In Cu(II),Co(II),Ni(II),Fe(II) complexes, the TGA curve showed lose in weight in the temperature range ~200-250 indicates the presence of coordinated water in the complex[14]. The thermograph curve of Cu(II) complexes shows loss in weight in the temperature range 70-250 ^{0}C clearly indicate that two coordinated water molecules..The complex decomposes from 600-800^{0}C. The thermograph curve of Co(II) complexes shows loss in weight in the temperature range 70-250 ^{0}C clearly indicate that two coordinated water molecules..The complex decomposes from 250-700^{0}C. The thermograph curve of Ni(II) complexes shows loss in weight in the temperature range 70-250 ^{0}C clearly indicate that two coordinated water molecules..The complex decomposes from 350-700^{0}C

CONCLUSION

In conclusion, according to the results obtained from FTIR and TGA, the new derivative of pyrazoline behave as bi dentate ligand toward the cobalt (II), nickel (II), cooper (II), and iron (II) ions. The active sites of the ligand in bonding with the metal ions are the two nitrogen atoms of pyrazoline ring which suffered red shift in their FTIR spectra. Metal complexes shows the octahedral symmetry with the general formula, $[M(L)_2(H2O)_2]$ and following structure.



Where M = Cu, Co, Ni, Fe and
R = -Br, -Cl ,-CH_3
R' = -Cl, -CH_3

ACKNOWLEDGEMENT

All Thanks and appreciation to Department of Chemical Sciences of SRTMUN and thanks to SAIF, Chandigarh, Punjab university for providing NMR spectral data and SAIF, Mumbai, IIT Bombay for providing FTIR and SICART, Gujarat for providing TGA data.

REFERENCES

1. H. Bauer, G. Piatert, German Patent 1981, (DOS) 3039311, Chem. Abstract. 95, 63146, (1981).
2. N.A. Evans, P.J.Waters, J. Soc. Dyers Colour 94, 252, (1978).
3. T. Murayama, N. Mater, New Processess Electrochem. Technol. 1, 192,(1981).
4. V.F. Poduzhailo, D.F. Pareyaslova, V.I. Shripkina, S.A. Verezubora, L.A. Andreeva, Zh. Prikl. Spektrosk. 1979 26, 357 Chem. Abstract 86, 162579, (1977).
5. Page S. Ruthenium compounds as anticancer agents. Educ Chem. 2012;10:26–29.
6. G. Khan, S. Merjver, Expert Opin Investing Drugs,4, 541, (2009)
7. B.S.Funiss, A.J. Hannaford, V.P. Rogers, W.G.Smith, A.R..Tat Hell, "Vogels text Book of practical organic chemistry," 4thEdn. ELB's Longman group Ltd, 268, 712, (1978).

8. W.C. Howell, M. Ktenas, and J.M. MacDonald, Tetrahedron Lett., 26, 1719,(1964).
9. P. Sohar, A. Csampai, P. Perjesi, V. Arkivoc, 114, (2003)
10. C.W. Wilson, J. Am. Chem. Soc., 61, 2303, (1938).
11. P. Sohar, P. Perjesi, K.W. Tornroos, S. Husebye, A. Vertes, G.Y. Vankoand, R.E. Bozak, J. Mol. Struct., 524, 297, (2000).
12. H Bin, S Shun-Jun, Bing J. Studies on transition metal ions recognition properties of 1-(2-benzothiazole)-3-(2-thiophene)2-pyrazoline derivatives, Dyes& Pigm 2007;73: 394-6
13. Khalil SM. Synthesis, Spectroscopic and Magnetic studies on metal complexes of 5-methyl-3-(2-hydroxy phenyl pyrazole. J Coord Chem. (2003).
14. M.V. Girgaonkar and S.G. Shirodkar, Research Journal of Recent Sciences, ISSN 2277 - 2502 Vol. 1(ISC-2011), 110-116 (2012)
15. Seema I Habib* S. Shah N. N., Mohammed A Baseer, Praffullkumar A ,Kulkarni J. Chem. Pharm. Res., 2011, 3(1):788-792.
16. Muhammad Imran, LiviuMitu, Shoomaila Latif, Zaid Mahmood, Imtiaz Naimat, Sana S. Zaman,J. Serb. Chem. Soc. 75 (8) 1075–1084 (2010)
17. P.Venugopal, K.Krishnakuty (1997). Synthesis and characterisation of cobalt-, nickel-, copper-and zinc (II) complexes of morin. Journal of the Indian Chemical Society, 74(7), 562-563.
18. H. L. Wong, L. S. M. Lam, K. W. Cheng, K. Y. K. Man, W. K. Chan, C. Y. Kwong, A. B. Djurišić, Low-band-gap, sublimable rhenium(I) diimine complex for efficient bulk heterojunction photovoltaic devices, Appl. Phys. Lett. 84, pp. 2557, (2004). doi:10.1063/1.1682676
19. B.B. Chavan, A.S. Gadekar, P.P. Mehta, P.K.Vawhal, A.K. Kolsure, A.R. Chabukswar, Asian Journal of biomedical and Pharmaceutical Sciences, volume 6, Issue 56, (2016)
20. G. G. Nitin, P. R. Rajput, V. W. Banewar and A. R. Raut, Int. J. Pharm. Bio.Sci., 3(3), 389-395,(2012).
21. P. K. Sharma, K. Kumar, P. Kumar, P. Kaushik, D. Kaushik, Y. Dhingra and K. R. Aneja, Eur. J. Med. Chem., 45, 2650-2655, (2010).
22. D.T. Sakhare, T.K. Chondhekar, S.G. Shankarwar and A.G. Shankarwar, Advances in Applied Science Research, 6(6):10-16, (2015).
23. Mahmoud Najim Al-Jibouri* and Taghreed M Musa, International Journal of Chemical Sciences Research | Vol 15 Iss 2
24. J. Joseph*, T. M. Sharmila and M.Sirajul Muneera, Journal of Chemical and Pharmaceutical Sciences, JCPS Volume 9 Issue 4,(2016).
25. Naveen Tanwer,1 Rajneesh Kaur,1,2 Devika Rana,1 Raman Singh,2 Kuldeep Singh ,Journal of Integrated Science and Technology , J Integr Sci Technol, 3(2), 39-41, (2015).
26. R. Kasımogullari, M. Bulbul, B.S. Arslan, B. Gokçe. Synthesis, characterization and antiglaucoma activity of some novel pyrazole derivatives of 5-amino-1,3,4-thiadiazole-2- sulfonamide. Eur. J. Med. Chem., 45, 4769-4773, (2010).

Table 1: List of synthesized α, β-unsaturated ketone with yield and melting point

Sr. No	Acetophenone ®	Aldehyde(R')		α, β- unsaturated ketone	Yield %	M.P.
1	Br	Cl	Benzaldehyde	$C_{15}H_{10}OBrCl$	82	120
2	Br	$-CH_3$		$C_{16}H_{13}OBr$	65	134
3	$-CH_3$	Cl		$C_{16}H_{13}OCl$	85	127
4	Br	-	Cinnamaldehyde	$C_{17}H_{13}OBr$	72	140
5	Cl	-		$C_{17}H_{13}OCl$	77	145
6	-Cl	Cl	Benzaldehyde	$C_{15}H_{10}OCl_2$	84	130

Table 2: List of synthesized ligand with structure, melting point, yield

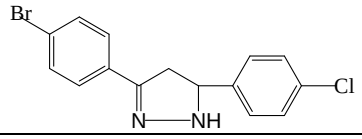
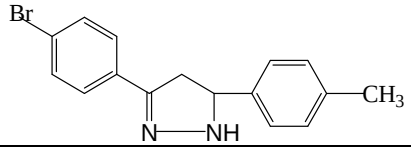
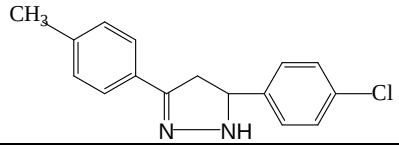
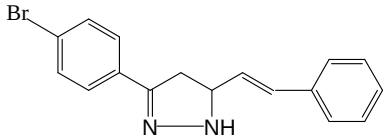
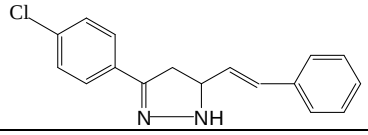
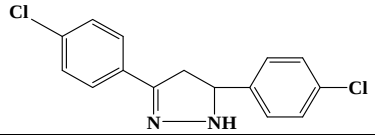
Sr. No.	Symbol	Molecular structure	Molecular weight	Melting point	Yield %
1	L2		336	110-115	72
2	L3		315	112-115	70
3	L4		271	107-109	71
4	L5		327	135-138	77
5	L6		283	140-141	75
6	L7		293	115-117	80

Table 3: List of synthesized metal complexes with structure, colour and melting point

Sr.No	Symbol	Metal complex	Colour	Melting point
1	L2C1	$[CuL_2(H_2O)_2]$ (L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)	Pale green	290-292
2	L2C2	$[CoL_2(H_2O)_2$ (L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)	peach	275-278
3	L2C3	$[NiL_2(H_2O)_2]$ (L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)	green	280-284
4	L2C4	$[FeL_2(H_2O)_2]$ (L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)	Faint peach	265-270
5	L3C1	$[CuL_2(H_2O)_2]$ (L=3-(4-bromophenyl)-5-(4-methylphenyl)4,5-dihydro-1*H*-pyrazole)	Pale green	220-225
6.	L4C2	$[CoL_2(H_2O)_2]$ (L=3-(4-methylphenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)	Pale green	231-233

Table 4: ^{1}H-NMR spectral data of ligand

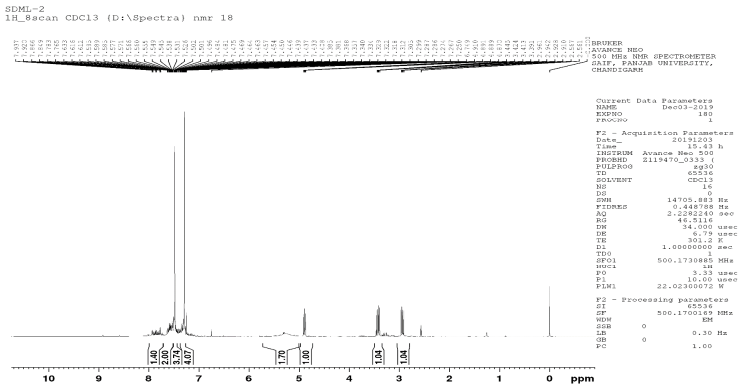
Ligand	δppm					
	Aromatic Protons	Pyrazoline ring N-H	Pyrazoline ring H1	Pyrazoline ring H2	Pyrazoline ring H3	-CH_3
L2	7.93-7.31(m,8H)	5.4 (s,1H)	4.91(dd,1H)	3.44(dd,1H)	2.96(dd,1H)	-
L3	7.88-7.40(m,8H)	5.62(s,1H)	4.82(dd,1H)	3.61(dd,1H)	3.12(dd,1H)	2.87(s,3H)
L4	7.93-7.13(m,8H)	5.50(s,1H)	5.31(dd,1H)	3.30(dd,1H)	2.57(dd,1H)	2.68(s,3H)
L5	8.03-6.51(m,8H)	5.44(s,1H)	4.48(dd,1H)	3.20(dd,1H)	2.39(dd,1H)	
L6	7.84-7.18(m,8H)	4.68(s,1H)	4.51(dd,1H)	3.22(dd,1H)	2.89(dd,1H)	
L7	7.97-7.26(m,8H)	5.35(s,1H)	4.82(dd,1H)	3.49(dd,1H)	2.98(dd,1H)	

Table 5: Infrared spectral data of ligand

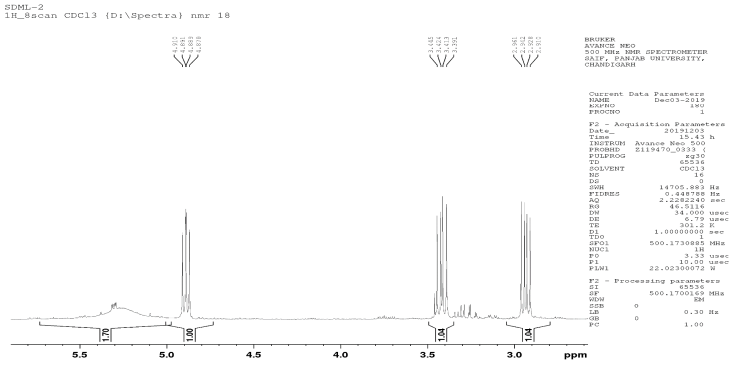
Ligand	υ(N-H)cm^{-1}	(C-H)aliphatic& Aromatic cm^{-1}	υ (C=N) cm^{-1}	C-Cl cm^{-1}	C-Br cm^{-1}
L2	3330.74	3137.42, 3063.17	1680.29	739.74	664.67
L3	3427.72	2966.21, 2917.61	1680.05	-	663.65
L4	3442.10	3029.27, 2919.00	1678.49	760.50	-
L5	3329.32	3058.56, 3026.35	1679.62	-	663.52
L6	3326.46	3058.70, 3026.69	1680.00	744.39	-
L7	3354.73	3098.23, 3029.65	1675.12	755.34	-

Table 6: Infrared spectral data of metal complexes

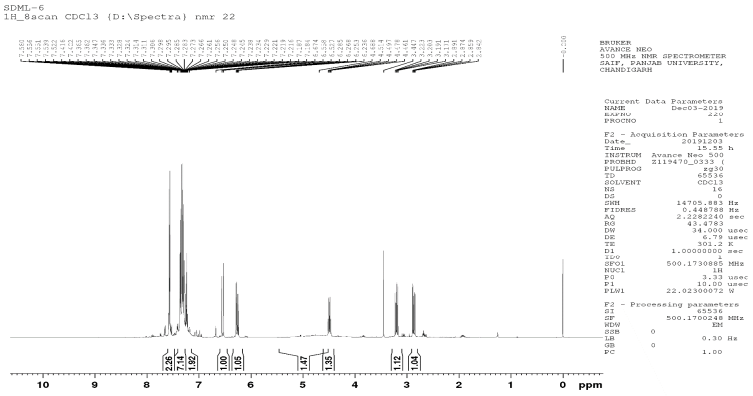
Metal complex	OH stretching cm^{-1}	υ (C=N) cm^{-1}	υ (C-N) cm^{-1}	υ (M-N) cm^{-1}	υ (M-O) cm^{-1}
L2C1	3477.16	1658.01	1587.75	516.81	480.81
L2C2	3427.83	1658.79	1586.97	533.43	450.74
L2C3	3421.92	1608.29	1580.00	542.87	484.50
L2C4	3344.78	1637.41	1585.32	551.47	466.66
L3C1	3335.26	1620.46	1590.13	516.61	433.70
L41C1	3421.75	1625.36	1589.41	517.87	437.06



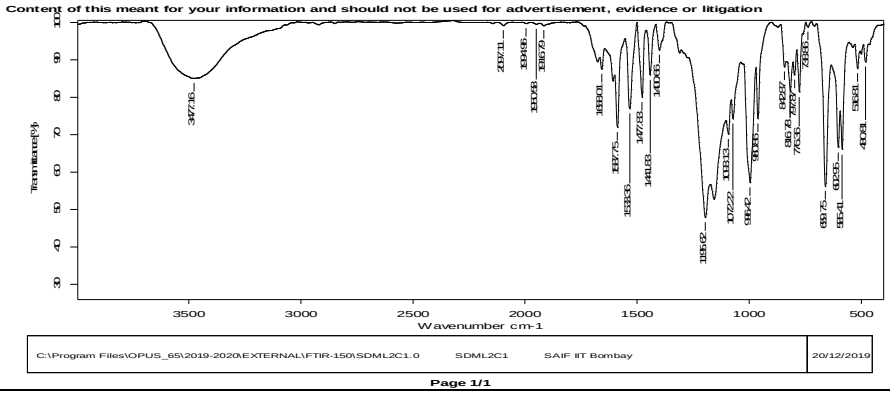
NMR Spectra For (L2)-3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole



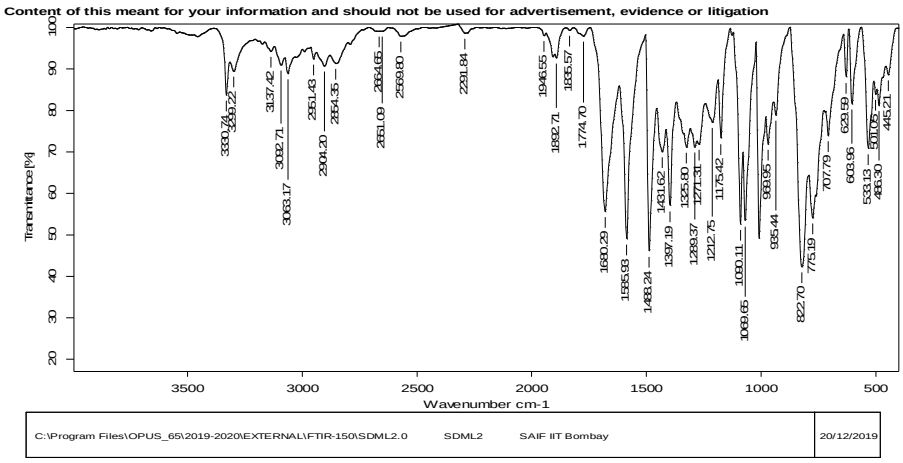
NMR Spectra For (L2)-3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole



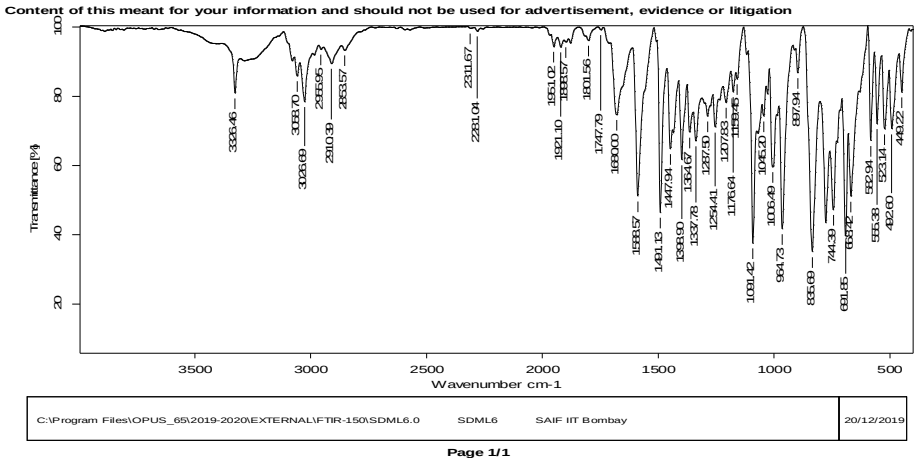
NMR Spectra For (L6)-3-(4-chloroophenyl)-5-(4-phenylethenyl)4,5-dihydro-1 *H*-pyrazole



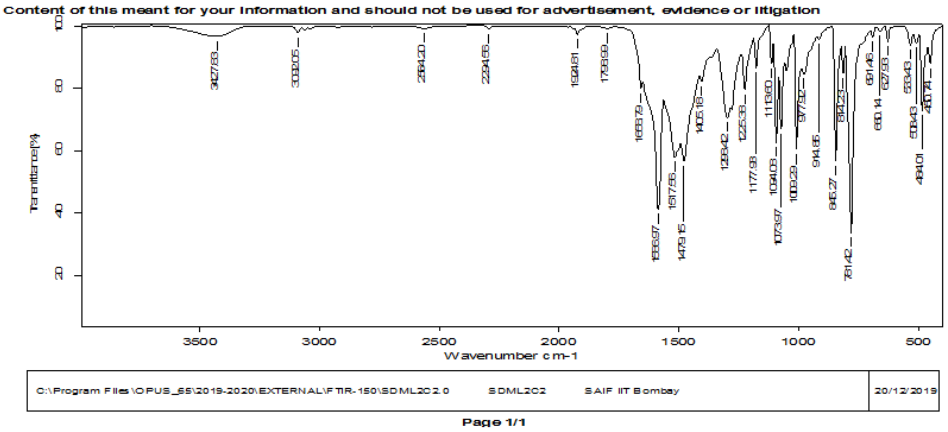
FTIR Spectra for $[CuL_2(H_2O)_2]$ metal Complex(L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1H-pyrazole



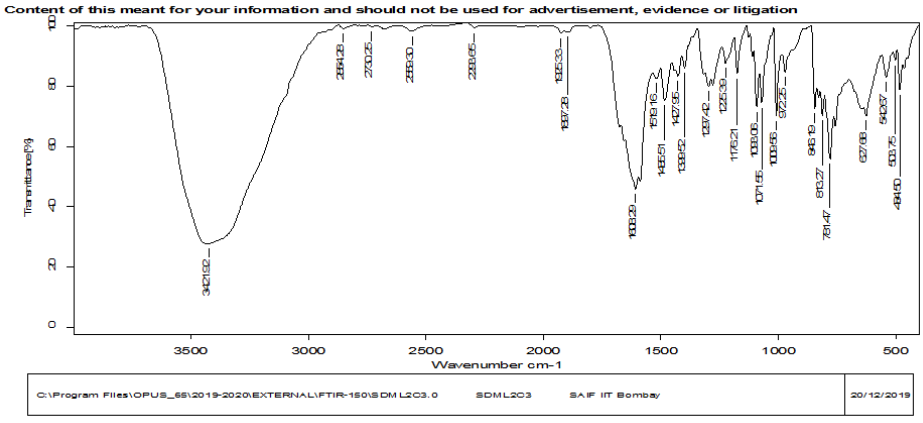
FTIR Spectra For (L2)-3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1H-pyrazole



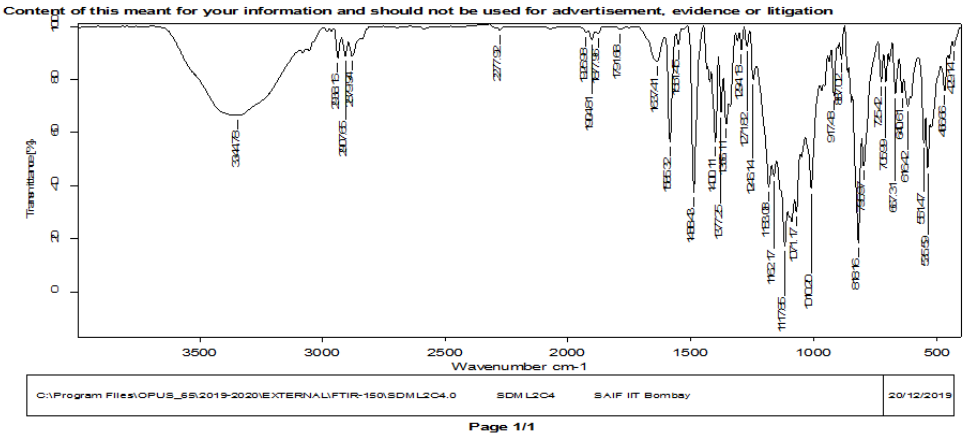
FTIR Spectra For (L6)-3-(4-chloroophenyl)-5-(4-phenylethenyl)4,5 -dihydro-1H-pyrazole



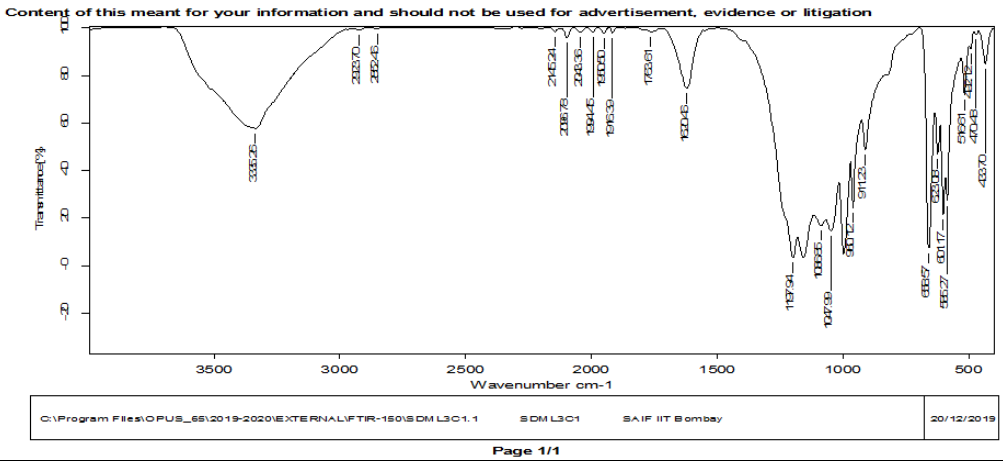
FTIR Spectra for $[CoL_2(H_2O)_2]$ metal Complex (L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)



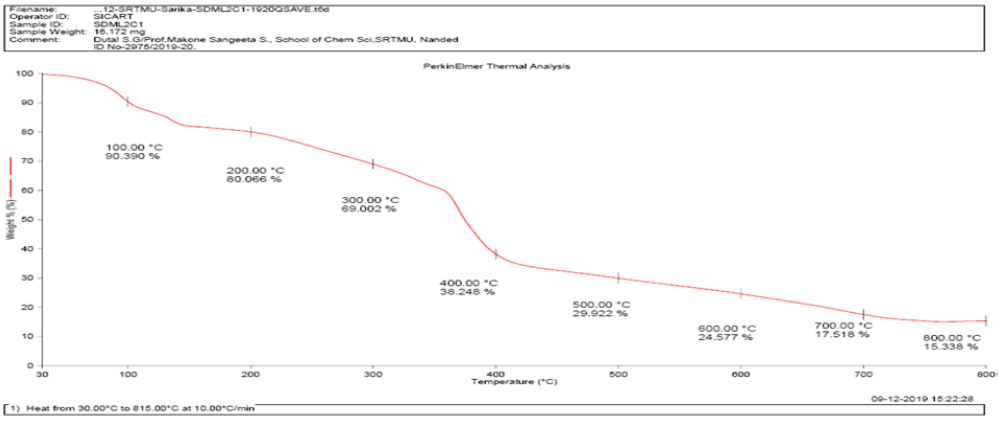
FTIR Spectra for $[NiL_2(H_2O)_2]$ metal Complex (L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)



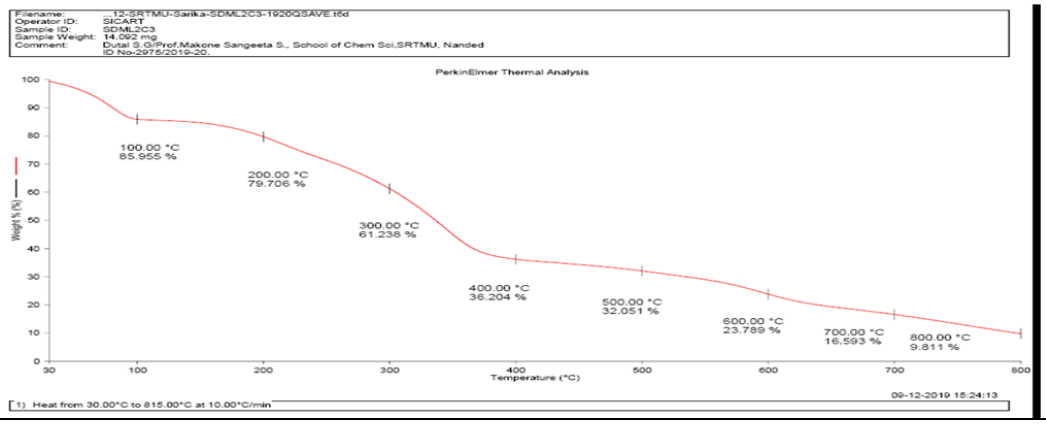
FTIR Spectra for $[FeL_2(H_2O)_2]$ metal Complex (L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)



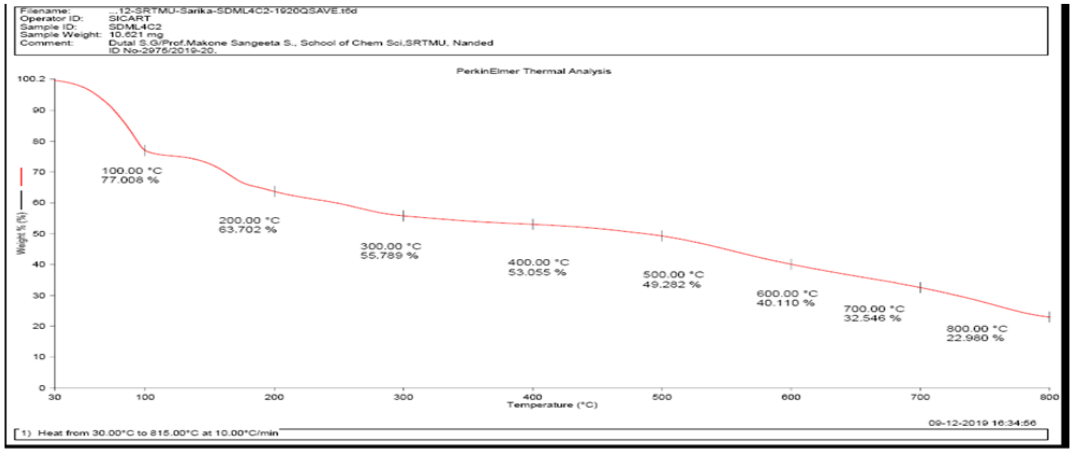
FTIR Spectra for $[CuL_2(H_2O)_2]$ metal Complex (L=3-(4-bromophenyl)-5-(4-methylphenyl)4,5-dihydro-1*H*-pyrazole)



TGA Spectra for $[CuL_2(H_2O)_2]$ metal Complex (L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)



TGA Spectra for $[NiL_2(H_2O)_2]$ metal Complex (L=3-(4-bromophenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)



TGA Spectra for [$CoL_2(H_2O)_2$] metal Complex (L=3-(4-methyphenyl)-5-(4-chlorophenyl)4,5-dihydro-1*H*-pyrazole)

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RESEARCH ARTICLE

Skill Requirement for Primary School Teachers - Indian Private Schools Scenario

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ABSTRACT

Primary classes are the formative years for children. It is very important for schools to find the right teachers to teach these young children. The skills required for a primary teacher are quite different from the skills required for a middle school or secondary school teacher. All teachers cannot adapt to all classes without appropriate skills. Undergraduation programs and B.Ed colleges are responsible for equipping the student teachers with skills required to become job ready competent teachers today. In the Indian Private School scenario, school authorities are the final decision makers who hire teachers. Hence it is very important to understand the skill requirements in their perspective. This study is an attempt to identify the expectations of school authorities from primary school teachers and to understand their view on how the teachers can be upskilled. Skilled teachers are an asset to the school and this in turn will transfer to the students and therefore the output will be creating a strong foundation in the young students. To find and analyze the skills expected by the school authorities while recruiting primary teachers. To identify the best way to upskill the primary school teachers. To identify the most important skills required for primary school teachers

Keywords: Teaching skills, Indian Teachers, Indian Private school owners, class management, Technology in teaching, Teaching competencies

INTRODUCTION

A skill is the learned ability to perform an action with determined results with good execution often within a given amount of time, energy, or both [1]. People need a broad range of skills to contribute to the modern economy [2]. A teacher's job is of a very demanding nature, as it deals with the minds of the young growing students. Teachers require multiple skills to handle students. Primary school teachers play an important role in shaping the educational path of students, and thus they will always be needed to establish a solid foundation of learning [3]. Primary school teachers are typically responsible for teaching children from first through fifth grades. They play an important role in developing a child's intellect and work habits, as primary school is the first time most children are in a strict educational environment. These teachers usually have one class of students that they will teach various subjects to for the entire school year. Subjects include mathematics, reading and writing, history and science. These teachers are responsible for instructing various groups of students. Hence there is a very high expectation on having various subject based knowledge and differential instructional skills.

Henry Adams, a famous Historian who descended from two US presidents once quoted – "Teachers affect eternity; No one can tell where their influence stops". Such is the effect of the teachers on students. The importance of primary school teachers can be found in the words of Dr.Abdul Kalam "Creativity is the key to success in the future, and primary education is where teachers can bring creativity in children". Based on various studies and researches there are a number of skills specific to teachers that can be enumerated as below. Communication, Class management, understanding teaching pedagogy, Content knowledge, Questioning Skill, Creativity, Patience, Technology, Conflict resolution, Collaboration, Enthusiasm, Dedication, Organizing, Presentation, Effective use of resources, Evaluation, Explanation, Stimulus variation, Leadership, Time management.

The current trend in Indian school education is the increase of private sector participation with an estimated 3 lakh private schools with 40% of the total student enrolment. Private enrolment in elementary schools is approximately 35%. The Annual Status of Education Report (ASER) shows that enrolment in private schools at the elementary in rural India has increased from 19% to 29% in the seven-year period from 2006 to 2013. Private schools play a very important part in building India's future workforce. Private schools are affiliated with various educational boards across India, from CBSE, ICSE, State boards and even International Boards like IGCSE. The private school authorities generally responsible for recruiting teachers are Correspondent or the owner, Principal and Vice Principal. Their expectations of what skills are required for a primary school teacher holds a value in the school job scenario. It is important to understand their outlook towards the B.Ed curriculum, Graduation and post-graduation programs and training methodology. Their views are equally relevant as is the need to understand Skill needs of MNCs and Other job sectors.

Scope of Study

In India, The National Council of Teachers Education mandates that the candidates who opt to pursue a teaching career in government schools and even in Private schools must have a B.Ed Course. Therefore B.Ed curriculum is supposedly designed as one stop to ensure all the skills necessary for teaching are being taught to the student teachers. Bearing this in mind this study has been undertaken to understand what are the top 4 skills the school authorities think are most important for a primary school teacher. According to the view of the school authorities, what are some of the skills that are not taught enough in the B.Ed Programmes.

RESEARCH METHODOLOGY

Descriptive research methodology has been used for this study.

Population: Finite known population - 60 Unique schools were taken for the study. From each school one key decision maker was taken for the study. The key decision makers were either School Correspondent or Principal or Vice principal - who are responsible for teacher recruitment in schools.

Sampling Method: Simple Random sampling
Sample size: 60
Date Source: Primary and Secondary
Data collection tool: Primary data collected through well-structured questionnaires
Tools used for statistical analysis: Mean, Ranking and Chi Square test.

RESULTS AND DISCUSSIONS

Reliability Statistics

Since the value of Cronbach's Alpha is 0.822, which is greater than 0.7, we can conclude that the data of our study is quite good enough for our research and is having great reliability. To determine the reliability and validity of the data Chronbach alpha test method was used. The test result showed .831 value, and hence data has been considered valid for further study.

Descriptive Statistics

Based on our survey it was found that the utmost respondents (72%) contributed towards this research are females. Most of the respondents (40.3%) opined that the number of primary teachers in the school (Grade 1 – Grade 5) were 1 to 5. The majority of the respondent (40.1%) state that the number of female teachers in primary grades (Grade 1 – Grade 5) were 6 – 10. Most of the respondents (75.8%) state that the number of male teachers in primary grades (Grade 1 – Grade 5)are zero. According to the study, around 51.6 % of respondents say that the approximate number of new teachers looking to hire year on year for primary grades (Grade 1 – Grade 5) are from 0 to 2. Based on the survey the respondents around 38.8% opined that demo classes are used for teacher's interview for recruiting primary grade teachers (Grade 1- Grade 5). On the basis of the research it is evident that the majority of the respondents (36.14%) think that B-Ed is the education qualification required for primary teachers(Grade 1 – Grade 5)

Skills required for primary grade teachers: Among the basic skills for primary grade teachers, we had selected the top 4 that is communication, class management, understanding teaching pedagogy and creativity with the highest mean values 10.062, 6.708 and 5.447

Skills not taught in B- Ed curriculum: Among the basic skills not that are not taught in the B- Ed curriculum, we had selected the top 4 skills that are communication, effective use of resources, organizing and class management with the highest mean values 7.932, 7.164 and 5.808.

Skills existing teachers could improve on: Among the basic skills that the existing teachers could improve on, we had selected top 4 skills that are communication, creativity, effective use of resources and understanding teaching pedagogy with mean values 8.955, 7.74, 7.26 and 4.59.

Methods of skill improvement for primary teachers: Among the basic skills for the improvement of primary teachers, we had selected the top 3 effective ways of conducting workshops, regular monitoring, structured lesson plan and demo by seniors with mean values 6.32, 6.064, 4.648 and 4.384.

Chi Square Analysis

To find the relationship between Gender and Impact level of factors on skill level of teacher

H_0: There is no significant association between Gender and Impact level of factors on skill level of teachers
H_1: There is significant association between Gender and Impact level of factors on skill level of teachers

Interpretation: Since Value of $P < 0.05$, we reject null hypothesis and accept alternative hypothesis. So it can be concluded that there exists a relationship between Gender and Impact level of factors on skill level of teachers.

Implications of the study

Main qualification required to be a primary teacher: Graduation and Bachelors in Education

Main skills School authorities are looking at while recruiting teachers: Communication, Class management, Creativity

Skills teachers do not get to learn in B.Ed College: Communication, Class management, Effective use of resources

Skills existing teachers still lack: Communication, Class management, Effective use of resources, Creativity, Understanding Teaching pedagogy

Most effective way of improving teachers' skills: Trainings & Workshops

Factor that impacts teachers' skill the most: No.of Trainings and workshops attended

CONCLUSION OF THE STUDY

Importance of Training for Primary School Teachers

Better Training leads to better processes and better Work. In today's ever-changing marketplace, the importance of job training has never been greater. Workforce training is an indispensable way to keep your organization competitive. Employees are human, most will have weaknesses or gaps in their professional skills. Primary School teachers create the base for the future generation. Effective primary education can create a confident future workforce. Training teachers on effective communication skills can improve the quality of teaching and bring a positive change in primary school education. Some of the communication skills specific to teachers are Communicating Empathy, Communicating to Parents, Interacting with Colleagues and Supervisors, ability to Identify Student Needs , Giving clear instructions and Providing crisp explanation. Designing training modules around these skills can prove to be effective and reduce the existing skill expectation gap.

RECOMMENDATIONS

B.Ed curriculum framework and B.Ed educators should incorporate effective modules on practical communication and not just a theoretical paper on communications. Creating real life situations for student teachers to learn and test their own communication skills. Evaluating communication as a practical skill and quantifying the level of communication student teachers achieve. Regular training and workshops to be conducted for existing teachers on various skills. Creating opportunity for the teachers to practice skills through various on job training methods

REFERENCES

1. https://en.wikipedia.org/wiki/Skill
2. https://www.onlinecollege.org/jobs/education/primary-school-teacher/#:~:text=Primary%20school%20teachers%20are%20typically,from%20first%20through%20fifth%20grades.&text=Primary%20school%20teachers%20usually%20have,and%20writing%2C%20history%20and%20science.
3. https://ficci.in/spdocument/20385/ey-ficci-report-education.pdf
4. 4.https://collegedunia.com/courses/bachelor-of-education-bed
5. https://www.teachhub.com/professional-development/2017/05/5-teaching-skills-all-educators-should-foster/ - Colleen Sullivan
6. skills by Dr.Passi, https://work.chron.com/important-teachers-good-communication-skills-10512.html
7. https://content.wisestep.com/communication-skills-importance-teachers-students-employees/

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.822	.831	60

Descriptive Statistics: Percentage Analysis

Content	Factors	Percentage (%)
Gender of respondents	Male	28
	Female	**72**
Number of primary teachers	1-5	11.3
	6-10	**40.3**
	11-15	14.5
	Above 15	33.9
Number of female primary teachers	1-5	14.5
	6-10	**37.1**
	11-15	16.1
	Above 15	32.3
Number of Male primary teachers	0	**75.8**
	1-5	24.2
	6-10	0
	11-15	0
	Above 15	0
Number of new teachers for primary grades	0-2	**51.6**
	3-5	45.2
	6-10	3.2
	Above 10	0
Interview modes	Telephone interview	7.15
	Written test	16.62
	Face to face interview	35.28
	Demo class	**38.84**
	Others	2.13
Education qualification	Graduation	30.12
	Early Childhood/Montessori training	10.04
	B-Ed	**36.14**
	Others	24.06

Skills required for primary grade teachers

Contents	Mean Value	Rank
Class Management	6.708	2
Communication	10.062	1
Understanding teaching pedagogy	5.447	3
Content knowledge	4.407	5
Questioning skills	1.261	9
Creativity	5.447	3
Patience	5.031	4
Enthusiasm	2.938	7
Dedication	3.354	6
Organizing skills	0.208	11
Presentation skills	2.093	8
Preparation skills	0.624	10
Effective uses of resources	4.407	5

Skills not taught in B- Ed curriculum

Contents	Mean Value	Rank
Class Management	5.808	3
Communication	7.932	1
Understanding teaching pedagogy	3.876	6
Content knowledge	5.22	4
Questioning skills	4.26	5
Organizing skills	7.164	2
Presentation skills	3.672	7
Preparation skills	1.74	8
Effective uses of resources	7.932	1
Lack of creativity	0.384	9
Assessment techniques	0.192	10
Current affairs	0.192	10

Skills existing teachers could improve on

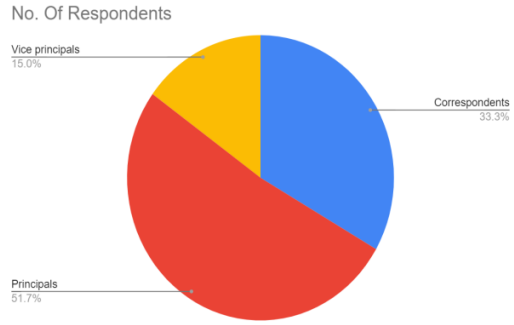
Contents	Mean Value	Rank
Class Management	4.35	5
Communication	8.955	1
Understanding teaching pedagogy	4.59	4
Content knowledge	2.655	6
Questioning skills	4.35	5
Creativity	7.74	2
Patience	2.415	7
Enthusiasm	2.91	5
Dedication	2.415	7
Organizing skills	4.11	5
Presentation skills	4.11	5
Preparation skills	1.935	8
Effective uses of resources	7.26	3
Differentiated teaching	0.24	9
Differentiated education	0.24	9

Methods of skill improvement for primary teachers

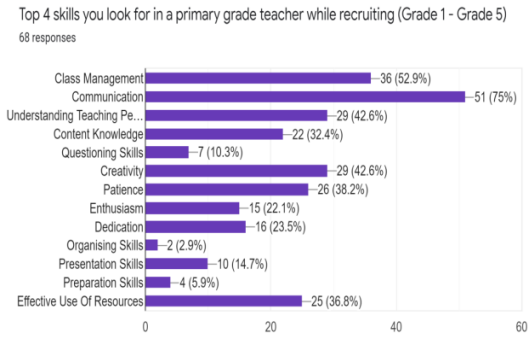
Contents	Mean Value	Rank
Workshops	6.32	1
Demo by senior	4.384	4
Advising	0.128	6
Regular monitoring	6.064	2
Co teaching	2.192	5
Structured lesson plan	4.648	3
Demo class by teachers	0.128	6
Mentoring	0.128	6

Chi square analysis: Gender and Impact level of factors on skill level of teachers

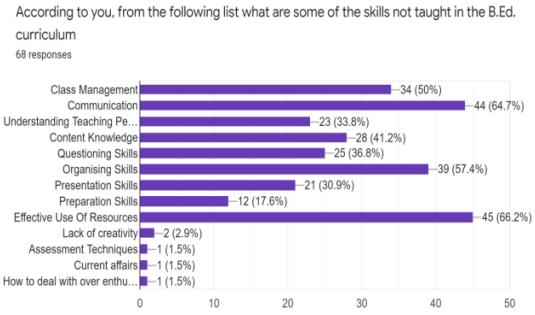
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.001[a]	8	.004
Likelihood Ratio	13.812	8	.001
Linear-by-Linear Association	2.503	1	.002
N of Valid Cases	60		



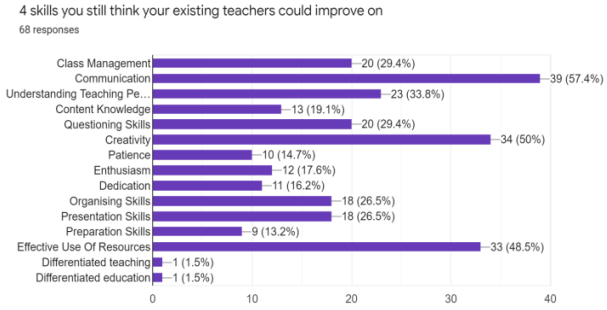
Descriptive Statistics



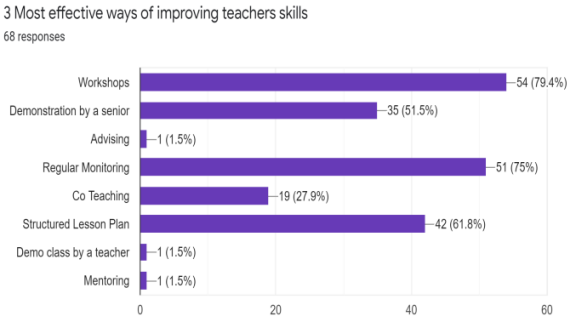
Skills required for primary grade teachers



Skills not taught in B- Ed curriculum



Skills existing teachers could improve on



Methods of skill improvement for primary teachers

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RESEARCH ARTICLE

Evaluation of Anticonvulsant Property of *Terminalia mollis* in Various Types of Experimentally Induced Seizures in Rats

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ABSTRACT

To evaluate the anticonvulsant and antioxidant properties of the ethanolic extract of *Terminalia mollis* using three animal models of peak electric shock (MES), pentylenetetrazole (PTZ) and strychnine nitrate (STN) to induce seizures in rats. In all three animal models MES, PTZ, STN, each model consisted of 4 groups, in which albino mice (n = 6) were used in each group. The first group was considered a control, the 2nd standard group used diazepam 4 mg/kg, the 3rd and 4th groups were the experimental groups treated with *Terminalia mollis* ethanolic extract (EETM) of 200 and 400 mg/kg respectively in three experimental animal models, all groups were treated for 14 days. On the final day, i.e. day 14 after the end of all drug administration in three animal models representing a total of 12 groups of rats, mice experienced convulsions for 3040 min. They were exposed to a shock of 150 mA with a convulometer using an ear electrode for 0.2 s in the MES model, 75 mg/kg intraperitoneally in the Pentylenetetrazole (PTZ) model and 2 mg/kg in the Model Strychnine (STN). Anticonvulsant activity was evaluated only after removal of posterior limb tone prolongation (HLTE) in the peak electric shock (MES) model and by measuring the duration of seizures and by measuring the duration of seizures and latency induced seizure threshold in the PTZ and STN experimental rat models.In the MES model, EECE at 400 mg/kg completely abolished HLTE in rats, similarly, at the same dose, an extended wait time to seizure onset was observed in tissues. Experimental animal models PTZ and STN. It was concluded that EETM showed effective anticonvulsant activity in these animal models because it eliminated HLTE in the MES model and delayed seizure threshold in the PTZ and STN models.

Keywords: Ethanolic extract of *Terminalia mollis* (EETM), Anticonvulsant activity, Antioxidant, Maximal electroshock (MES), Pentylenetetrazole(PTZ), Strychnine (STN), Diazepam.

INTRODUCTION

Epilepsy is a group of disorders characterized by recurrent spontaneous seizures that appear to be the result of complex processes involving several neurotransmitters, namely the glutamatergic, cholinergic and gabaergic [1]. Expression of synchronous flares alternating with periods of normal electrical activity [2]. Glutamate and gama amino butyric acid (GABA) are quantitatively the most important excitatory and inhibitory neurotransmitters in the brain mammal [3]. Therefore, these two neurotransmitters are reported to be important targets to induce antiepileptic action. Approximately 30% of patients with partial epilepsy and 25% of patients with generalized epilepsy do not recover completely with allergen administration [4]. Many of these patients often take some form of medical treatment to control their seizures. Therefore, there is an unmet need to identify new molecules with anti-epileptic properties. In our study, we selected herbal medicine and it could be one of the sources of new anti-epileptic treatments [5].

Terminalia species (Combretaceae) are widely used in traditional medicine to treat a wide range of diseases in the tropics and subtropics. It is a plant tree 11 to 26 meters high with dark gray bark deeply cracked. *Terminalia mollis* is a plant species used to treat gonorrhea, as an antifungal [6] malaria disease [7], and as an alternative in the treatment of HIV in Africa 8. In Tanzania, it is commonly used to treat malaria and as an adjuvant treatment for patients infected with HIV, as well as to treat diarrhea and bacterial infections [8,9] and has antiproliferative activity [7]. Several important natural compounds on the skin Therapeutic plan have been isolated from *Terminalia* krill such as alkaloids, flavonoids, carbohydrates, tannins, saponins and steroids) and all of these compounds can act as very powerful and reliable drug molecules for the treatment of various disorders. This study was performed to assess the possible anticonvulsant activity of *Terminalia mollis* extract using different in vivo models such as maximal electroshock (MES), as well as pentylenetetrazole (PTZ) and strychnine (STN) induced seizures.

MATERIALS AND METHODS

Drugs & Chemicals

Ethanol, Aceticanhydride, Conc. H_2SO_4, $CHCl_3$, Magnesium, Con. HCl, $FeCl_3$, Dragendroff's reagent, Kedde's reagent, Absolute alcohol, Chloroform, N-butanol, Acetic acid , Ninhydrin, Nacl, Trichloroacetic acid, Thiobarbituric acid, DTNB, EDTA, CMC & Diazepam. PTZ & Strychnine (Yarrow chemicals).

Plant collection

The root of *Terminalia mollis* collected during Sep 2019 from Tirumala hills, Andhra Pradesh, India. It was identified and authenticated by Prof.Dr.K.Madhava Chetty, Dept.Of Botany, University, Tirupati, and Andhra Pradesh, India. The voucher (VoucherNo:0421) specimen was maintained in our laboratory for the future references.

Experimental animals

Adult male Wistar rats, weighing 150-180g, were procured from the animal house of CES College of pharmacy, Chinnatekur, Kurnool (Reg., no.1278/ac/09/CPCSEA). The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 hr light and 12 hr dark day night cycle) and had free access to commercial pellet diet with water ad libitum. The temperature was maintained at 25 ± 10C with relative humidity (50 ±15%). The study was approved by the institutional animal ethical committee (IAEC/CESCOP/2019-08).

Acute toxicity study

The acute toxicity of 90% ethanolic root extract of *Terminalia mollis* (EETM) was determined as per The Organization for Economic Cooperation and Development (OECD) guideline no. 423 (Acute Toxic Class Method). It is observed that the plant extract was not mortal even at the dose of 2000mg/kg. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of

this dose were chosen to further study[10].Acute oral toxicity obtained results were indicated that *Terminalia mollis root* extract doses up to 2000 mg/kg did not produce any symptoms of acute toxicity and none of the rats died till 72 hours. On observation of rats up to 14 days none of animal died.

Grouping of animals

In each individual animal model i.e; MES, PTZ, STN having 4 groups, and each group had six rats. This grouping was common to all 3 animal models. Group I rats received sodium carboxy methyl cellulose (SCMC), Group II received Phenytoin / diazepam, Group III received EECE 200 mg/kg and Group IV received EECE 400 mg/kg. In Maximal electro shock seizure (MES) model, Animals exhibit hind limb tonic extension (HLTE) and the percentage of animals protected against HLTE were considered when it is abolished in 10sec and hind limb extension of body.In Pentylenetetrazole (PTZ) and Strychnine(STN) models**,** Latency of seizure threshold, duration of seizures, % of animals protected against seizures, % of animals protected against lethality were recorded within a thirty minutes durationafter intraperitoneal injection of (PTZ)and (STN).

Induction of seizures in rats

1) Maximal Electroshock Seizure (MES) model [11]

Test was performed to induce seizures in Albino mice of either sex. Mice were subjected to shock of 150 mA by convulsion meter through ear electrodesfor2seconds on 14th day after 30 minutes of administering the last dose of vehicle, diazepam and extracts. The number of animals exhibiting hind limb tonic extension (HLTE) seizures and the percentage of animals protected against HLTE were recorded .

2) Pentylenetetrazole(PTZ) and Strychnine(STN) models [11]

Albino rats of either sex were used to induce seizures. On the last day i.e.,14thday,30min after administration of the last dose of the vehicle, diazepam and the test extracts ,seizures were induced in rats in both models by Intraperitoneal injection of Pentylenetetrazole (PTZ)75 mg/kg, and Strychnine (STN)-induced seizure with the dose2.5mg/kg. The latency to PTZ and STN-induced seizures threshold, the duration of seizures, percentage of animals protected against seizures and percentage of animals protected against lethality were recorded within a thirty minutes duration[9] after intraperitoneal injection of (PTZ) and (STN) .

Statistical analysis

Data were presented as percentage (%) protection and mean ±SEM and were analyzed by one-way ANOVA followed by Dunnett's test for multiple comparisons using Graph pad prism version 5.03. Results were considered significant at $p<0.05$.

RESULTS

The percentage yield of ethanol extract of entire plant of *Terminalia mollis* was found to be 3.25 %w/w respectively.

Acute toxicity study

The ethanolic root extract of *Terminalia mollis* was observed to safe up to 2000mg/kg by oral route. Animals were found to be well tolerated after 24 hours. There were no deaths or symptoms of toxicity. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for biological study.

DISCUSSION

The study results indicate that the EETM (200 and 400 mg/kg) have anticonvulsant property in an animal models MES and PTZ-induced seizure. Antiepileptic drugs which abolish tonic extension occurred by MES acts by inhibiting

spread of seizures. Drugs that either prevents or delay seizure occurrence caused by PTZ, act by elevating the seizure threes hold[12]. In our study, in the maximal electro shock seizure (MES) test, 100% of the controlled rats exhibited hind limb tonic extensions (HLTE) seizure. The MES is a standard procedure which evaluates the ability of the testing materials to protect against HLTE. The seizure features in MES are similar for all laboratory animals and human except for the time scale[13] .The standard drug diazepam (4mg/kg) and the EECE (200 and 400mg/kg) exhibited significant anticonvulsant activity and provided protection against electroshock induced HLTE respectively. In the MES, protection against HLTE predicts the anticonvulsant activity of the tested compounds. More over protection against HLTE in MES- induced seizure indicates the efficiency of *Terminalia mollis extract* to either stop or to slowdown the discharge of the seizure within the brain stem substrate[14].Seizure induced by MES can be blocked either by inhibiting the voltage-dependent Na^{+}channels or by blocking glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA)receptors[15]. Since *Terminalia mollis* extract showed anti-epileptic activity in the MES, itmayactbythe same mechanism of action [16].The significant anticonvulsant activities *Terminalia mollis* extract may be due to the presence of many potent compounds or phyto constituents such as flavonoids, phenols and terpenes [17]. In PTZ test, diazepam (4mg/kg), *Terminalia mollis (*200 and 400 mg/kg) extracts exhibited a significant anticonvulsant effect. *Terminalia mollis 400 mg/kg* was found to be more effective than *200mg/kg*.These results gives us evidence that ethanolic extract possesses anticonvulsant activity. The ability of ethanolic extracts to delay the onset of convulsions and/or shorten the duration of convulsions was considered an evidence of anticonvulsant activity.

In studies it is known that compounds which are effective in suppression of PTZ-induced clonic seizures partially overlapped with the group of compounds effective against MES [18]. Inthis regard, diazepam was found to be more effective against PTZ than MES seizures . PTZ is a GABA-A receptor antagonist. Accordingly, PTZ produces seizures by blocking the major GABAergic inhibitory pathways in the central nervous system[19]. Standard antiepileptic drugs such as diazepam are thought to produce their effects by enhancing GABA-mediated inhibition in the brain [20]. Moreover, activation of the N-methyl-d-aspartate (NMDA) receptors is also involved in the initiation and propagation of PTZ-induced seizures .In this regard, drugs that block glutamatergic excitation mediated by NMDA receptor shave demonstrated anticonvulsant activity against PTZ-induced seizures[21]. Seizures induced by PTZ can also be blocked by reducing T-type Ca^{2+} currents [22]. Therefore, the anti convulsant activities of *Terminalia mollis* extracts against PTZ seizures might be due to an enhancement in release of inhibitory neurotransmitter GABA in the central nervous system, inhibiting T-type Ca^{2+} currents or blocking the glutamatergic neurotransmission mediated by NMDA receptors ,which were not tested in this study. However, NO a molecule that can freely access with O_2free radicals in the brain and reduce the oxidative stress induced damage by blocking free radicals [23] . Probably, in PTZ rats the reduced level of NO is resulted from free radicals production at seizure time and its consumption due to its cleaning effect. The enhanced level of NO in treated group with EETM extract is also due to its antioxidant effect by which eliminates O_2.- radicals, and consequently prevents lipid peroxidation and oxidative stress-induced injury that leads to increased level of NO.STN directly antagonizes the inhibitory spinal reflexes of glycine [24]. EETM exhibit its anticonvulsant effect on STN-induced convulsions thus indicating its glycine independent activity.

CONCLUSION

Present study evaluated protective effect against seizures induced by MES, PTZ and STN and anti-oxidant activity of ethanolic root extract of *Terminalia mollis*. The observed antioxidant and anticonvulsant activities are due to the presence of considerable amount of flavonoids and phenolics in the extract of *Terminalia mollis*. Ethanolic extract of 400mg/kg *Terminalia mollis* compared to 200mg/kg showed good anticonvulsant activity in MES, PTZ as well as STN induced convulsions may be due to the involvement of GABAergic and glutaminergic transmission and through glycine inhibitory property. However, further studies are needed to develop the exact underlying mechanism of anticonvulsant action of possible constituents of the plant after isolation of bioactive compounds.

REFERENCES

1. Sander JW, Shorvon SD. Epidemiology of the epilepsies. J. Neurol. Neurosurg. Psychiat. 1996, 61, 433-443.
2. Dichter M. Basic mechanisms of epilepsy: targets for therapeutic intervention. Epilepsia 1997; 38 (Suppl. 9): S2– S6.
3. Rang HP, Dale MM, Ritter JM, Moore PK, Pharmacology. Churchill Livingstone, Edinburgh 2007.
4. Richens A, Perucca E. Clinical pharmacology and medical treatment. In: Laidlaw J, Richens A, Chadwick, D. (Eds.), A Textbook of Epilepsy. Churchill Livingstone, Edinburgh 1993: 495–560
5. Mikael E. Pedersen, Henrik T. Vestergaard, Suzanne L. Hansen, Sekou Bah, Drissa Diallo, Anna K. Jäger. Pharmacological screening of Malian medicinal plants used against epilepsy and convulsions. Journal of Ethnopharmacology 2009; 121: 472–475.
6. P. Mosoko, J. Picard, J.N. Eloff, Antifungal activities of six South African *Terminalia* species (Combretaceae), Journal of Ethno pharmacology, Volume 99, Issue 2, 3 June 2005.
7. *Raymond muganga, L. Angelot, M. Tits, M. Frederich,* Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria, Journal of Ethno pharmacology, Volume 128, Issue 1, 2 March 2010.
8. Sheila MgoleMaregesi, Luc Pieters, Olipa David Ngassapa, Sandra Apers, Rita Vingerhoets, Paul Cos , Dirk A. Vanden Berghe, Arnold J. Vljetinck, Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities, Journal of Ethnopharmacology, Volume 119, Issue 1, 2 September 2008.
9. *YKE Ibrahim, BA Tytler, YK Ogunsola,* Phytochemical and antimicrobial screening of crude extracts (aqueous and ethanolic) of Terminalia mollis linn (combretaceae) root, Nigerian Journal of Pharmaceutical Research, Vol 5 No 1 (2006).
10. IsholaIO,olayemiSO,YemitanOK,Ekpemandudiri NK.Mechanisms of anticonvulsant and sedative actions of the ethanolic stem-bark extract of Ficussur Forssk (Moraceae) in rodents.PakJBioISci2013;16:1287-1294.
11. Ayyanna C , B.Ramesh, Sree Sudha TY., PugazhenthanThangaraju,Evaluation of anticonvulsant and antioxidant properties of *Cyperus esculentus* Linn. in various types of experimentally induced seizures in rats. International Journal of Green Pharmacy. 2020; 14(4):381-87.
12. BumEN, Schmutz M, Meyer C,Rakotonirina A, Bopelet M,Portet C. Anticonvulsant properties of them ethanolic extract of Cyperus articulatus. J Ethnopharmacol2001; 76:145–150.
13. Ibrahim G, Abdulmumin S, Musa K, Yaro A. Anticonvulsant Activities of Crude Flavonoid Fraction of the Stem Bark of Ficussy comorus (Moraceae).J Pharmacol Toxicol 2008;3:351-356.
14. NagarajaT,Mohamood R,Krishna V,Thippeswamy B, Veerapur V.Anticonvulsant activity of Erythrina mysorensis bark extract in an animal model of epilepsy.JPharmacolPharmacother2012;3:62–64.
15. Luszczki JJ,Lucyna A,Czuczwar SJ.1-methyl-1,2,3,4- tetrahydroisoquinolineenhancesthe anticonvulsant action of carbamazepine and valproate in the mouse maximalelectroshock seizure model. Neuro pharmacol2006;50:133–142.
16. AliM, Chaudhary N.FicushispidaLinn.:A review of its Pharmacognostic and ethnomedicinal properties. Pharmacogn Rev2011; 5:96–102.
17. Williams CA,Goldstone F,Greeham J.Flavonoids, cinnamic acid sand coumarins from the different tissues and medicinal preparations of Taraxacum officinale.Phytochemistry1996;42:121–127.
18. Meldrum BS. Do preclinicalseizure models preselect certain adverse effects of antiepileptic drugs. EpilepsyRes2002;50:33-40.
19. Gupta G,Afzal M,David S,Verma R,Candaswamy M, Anwar F.Anticonvulsant activity of Morusalbaandits effect on brain gamma-aminobutyric acid level in rats. Pharmacog Res 2014;6:188-189.
20. Mac Donald RL, Kelly KM. Anti-epileptic drug mechanisms of action.Epilepsia1995;36:S2–S12.
21. Meldrum BS Update on the mechanism of actionofantiepilepticdrugs.Epilepsia1996;37:S4–S11.
22. Kuno M, Weakly JN. Quantal components of the inhibitory synaptic potentials in spinal motor neurons of cat.JPhysiol1972;224:287–303.
23. Sudha K, Rao AV, Rao A (2001). Oxidative stress and antioxidants inepilepsy. Clin. Chem., 303: 19-24.
24. Chen LW, Wang YQ, Wei LC, Shi M, Chan YS 2007. Chinese herbs and herbal extracts for neuroprotection of dopaminergic neurons and potential therapeutic treatment of Parkinson's disease. *CNS NeurolDisord Drug Targets 6*: 273-281.

Table: 1 Phytochemical Constituents

S.NO	TEST	INFERENCE
1	Lieberman's test	-
2	Salwoski test	-
3	Shinoda test	+
4	Ferric chloride	+
5	Dragendroff's test	-
6	Brontranger's test	-
7	Kedde's test	-
8	Legal's test	-

+ indicates present - indicates absent

ANTICONVULSANT ACTIVITY

Table No: 2 Effect of EETM in MES Induced Convulsions in Rats

Groups	Drug Treatment	Flexion (Sec)	Extensor (Sec)	Clonus (Sec)	Stupor (Sec)	Recovery (Sec)	Percentage Protection
I	Control (3% w/v SCMC)	6.64±0.26$^{\#\#\#}$	13.4±0.84$^{\#\#\#}$	15.20±1.24$^{\#\#\#}$	30.86±0.82$^{\#\#\#}$	Recovery	44
II	Diazepam (4 mg/kg/i.p.)	3.46±0.46 ***	0	10.2±1.45 ***	12.26±0.45 a***	Recovery	100
III	EECE (200 mg/kg/p.o.)	4.68±0.34b ***	4.17±0.16 **	7.44±0.26 ***	18.34±2.34 b**	Recovery	70
IV	EECE (400 mg/kg/p.o.)	3.88±0.38b ***	2.19±0.24 ***	5.62±0.42 ***	15.24±1.72b**	Recovery	92

Where n=6 the observation are Mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 as compared to control. All the data were analyzed by using one way ANOVA followed by Dunnett's test.

Table 3: Effect of EETM on PTZ Induced Convulsions in Rats

Groups	Drug treatment	Latency (sec)	Onset of Jerky movements (sec)	Onset of Straub's tail (sec)	Onset of Clonic convulsions (sec)	No.of animals alive	%Inhibition
I	PTZ 75mg/kg/I.P	34.17 ± 4.061$^{\#\#\#}$	62.50± 3.819$^{\#\#\#}$	29.50± 2.930$^{\#\#\#}$	37.67 ± 3.648$^{\#\#\#}$	2	33%
II	Diazepam4mg/kg/I.P	149.3± 4.425***	162.5± 3.819***	111.2± 5.712***	162.5± 3.819 ***	6	100%
III	*EETM*200 mg/kg/P.O	113.7± 3.528**	118.8± 5.062**	67.83± 3.208**	103.7± 4.863**	4	66%
IV	*EETM*400 mg/kg/P.O	143.8± 4.135***	153.8± 4.729***	99.67± 2.883**	151.7± 4.410 ***	5	83%

Where n=6 the observation are Mean ±SEM.*P<0.05,**P<0.01and ***P<0.001 as compared to control All the data were analyzed by using one way ANOVA followed by Dunnett's test. *EETM*–Ethanolextract of *Terminalia mollis*

Table 4. PTZ Induced Seizure-Antioxidant Studies

Groups	Drug treatment	Lipid peroxidation (nm)	Glutathione (nm)	Catalase (nm)	GABA (nm)
I	PTZ 75 mg/kg/I.P	0.4857± 0.02130$^{###}$	0.1821± 0.00853$^{###}$	0.2938± 0.02024$^{###}$	0.6763± 0.01768$^{###}$
II	Diazepam4mg/kg/I.P	0.2738± 0.01261***	0.4302± 0.00945***	0.7588± 0.01834***	0.3457± 0.02098***
III	*EETM*200 mg/kg/P.O	0.3531±0.01478 **	0.2721± 0.00968**	0.4163± 0.02483**	0.4635± 0.01616**
IV	*EETM* 400 mg/kg/ P.O	0.2558± 0.01197***	0.3792± 0.01726***	0.6941± 0.02184***	0.3722± 0.01654***

Wheren =6 the observation are Mean±SEM. *P<0.05, **P<0.01 and ***P<0.001 in comparison to the control all of the data was evaluated with a one – way ANOVA and Dunnett's test. *EETM* –Ethanolic extract of *Terminalia mollis.*

Table 5: Effect of EETM on Strychnine Induced Convulsions

Groups	Drug treatment	Latency(sec)	Onset of Jerky movements (sec)	Onset of Straub'stail (sec)	Onset of Clonic convulsions (sec)	No.	%Inhibition
I	STN 2 mg/kg/I.P	86.00± 7.095$^{###}$	28.67± 3.106$^{###}$	30.00± 2.978$^{###}$	42.83± 3.600$^{###}$	2	33%
II	Diazepam 4mg/kg/I.P	153.3± 5.725***	12.33± 1.706***	113.8± 5.671***	20.67± 2.186***	6	100%
III	*EETM* 200 mg/kg/P.O	118.0± 5.645**	17.83± 2.088**	68.33± 3.879**	25.58± 2.647**	4	66%
IV	*EETM* 400 mg/kg/P.O	141.9± 5.294**	16.17± 1.815***	98.67± 4.716**	22.70± 2.423***	5	83%

Where n=6 the observation are Mean±SEM. *P<0.05, **P<0.01 and ***P<0.001 in comparison to the control all of the data was evaluated with a one-way ANOVA and Dunnett's test. *EETM*–Ethanolic extract of *Terminalia mollis.*

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RESEARCH ARTICLE

Antibacterial Activity of *Merremia tridentata* (L.) Hallier.F.

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ABSTRACT

The medicinal plants are the pharmacologically effective source to counteract various infectious diseases. *Merremia tridentata* (L.) Hallier.f. belongs to the family Convolvulaceae is a medicinal plant widely used in the ayurvedic system of medicine. The present study has been carried out to know the antibacterial activity of different extracts of *M. tridentata.* The extracts obtained from the aerial parts, root and flowers of *M. tridentata* have been tested against *Staphylococcus hominis, S. cereus, S. aureus, Escherichia coli* and *Bacillus subtilis* and the results are discussed. The extracts were observed to be more effective against Gram Positive strains of bacteria studied than the Gram Negative *E.coli*. In general, the maximum zone of inhibition (20 mm) was noticed for *S. cereus* in the methanolic extract obtained from the aerial parts of *M. tridentata.*

Keywords: Antibacterial activity, *Merremia tridentata,* Inhibition zone.

INTRODUCTION

Infectious diseases are still a major health issue, especially in developing countries, leading to the death of millions of people, despite enormous improvements in health care systems (York *et al*., 2011) This is primarily due to the acquired bacterial resistance to antibiotics (Chopra, 2000). These organisms trigger life threatening ailments and even may contribute to death (Daszak *et al*., 2011). Many bacteria have revealed resistance to synthetic antimicrobial drug e.g. resistance to *penicillin* by *Staphylococcus aureus* (Chain and Abraham, 1940). Eventhough pharmaceutical companies have produced a number of new antibacterial against in the past years, resistance to these drugs has increased and has now become a global concern (Adwan and Mhanna, 2008). Further, the global emergence of multi-drug resistant bacteria is increasingly limiting the effectiveness of current drugs and significantly causing failure in treatment (Hancock, 2005). Due to the increase of resistance to antibiotics, there is a pressing need to develop new

and innovative antimicrobial agents. Among the potential sources, plants have long been investigated as they contain many bioactive compounds that can be of interest in therapeutic purpose. For this reason the search is ongoing for new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources of antimicrobial agents (Bhavnani and Ballow, 2000). In the present study antibacterial activity of methanolic, chloroform and petroleum ether extracts from the aerial parts, methanolic and petroleum ether extracts from root and methanolic and chloroform extracts from flower extracts of *M. tridentata*.

MATERIALS AND METHODS

Antibacterial activity of aerial parts, root and flower was investigated by the disc diffusion method described by Irobi *et al*., 1994. Luria Bertani medium (LB) was used for antibacterial susceptibility tests. The LB medium was prepared by pouring 15 ml of molten media into the sterile Petri plates. The plates were allowed to solidify and inoculum suspension was swabbed uniformly on the medium and allowed to dry for 5 min. 30 µl of aerial, flower and root extracts (petroleum ether, chloroform and methanolic) containing 1.5mg/ml concentration was loaded on 6 mm sterile disc. The standard antibiotic disc Gentamycin (120mcg/disc) was placed on the surface of the plates. The plates were kept for incubation for 24 hrs at 37°C. The zone of inhibition was measured around the discs containing samples and standard. Aerial part, root and flower of *M. tridentata* were dried under shade for about a week. The dried material was ground to coarse powder. This powder was soaked in 100 ml each in petroleum ether, chloroform, and methanol for 3 days with occasional shaking and stirring. The solvent was filtered through filter paper (Whatmann No.1). After filtration the solvents, allowed to dry in hot air oven and the extracts were obtained. Condensed extracts were they weighed and stored in air-tight containers. The extract so obtained was then used for testing *in-vitro* antibacterial activity.

RESULT AND DISCUSSION

Antibacterial activity of different solvent extracts from aerial parts, methanolic and petroleum ether extract from root and methanolic and chloroform extract from flower has been tested against *Staphylococcus hominis*, *S. cereus*, *S. aureus*, *Escherichia coli* and *Bacillus subtalis* (Table 1, 2 & 3) The extracts were more effective against gram positive strains of bacteria than against gram negative E. coli. This is in accordance with the earlier work reported in this plant (Neyanila *et al*., 2013). The inhibition zone of methanolic extract of aerial parts ranged from 6-20 mm. The maximum inhibition was noted when methanolic extract was used against *S. cereus* (20 mm). In an earlier work in the related species *M. emergenata*, all the four extracts (aqueous, petroleum ether, chloroform and acetone extract) showed very good growth inhibition against the test organisms (oral bacteria). Further acetone extract alone showed maximum inhibitory action against the test microorganisms i.e. 24% for *Staphylococcus epidermidis*, 20% for against *Lactobacillus rhamnosus*, 11.11% for against *Streptococcus mutans*, 3.57% for *Escherichia coli* and 1.66% for *Staphylococcus aureus* (Diwan, *et al*., 2012).

CONCLUSION

In the present it can be concluded that antibacterial activity exhibited by various extracts of *M. tridentata* used in ayurvedic system of medicine for the treatment of various infectious disease. The extracts were observed to be more effective against Gram Positive strains of bacteria studied than the Gram Negative *E.coli*. In general, the maximum zone of inhibition was noticed for *S. cereus* in the methanolic extract obtained from the aerial parts of *M. Tridentata*.

REFERENCES

1. Adwan, G. and Mhanna, M. 2008. Synergistic effects of plant extracts and antibiotics on *staphylococcus aureus* strains isolated from clinical specimens. *Middle-East J. Sci. Res.*. 3:134–139.

2. Bhavnani, S.M. and Ballow, C.H. 2000. New agents for Gram-positive bacteria. *Curr Opin Microbiol*. 3: 528-534.
3. Chain, E. and Abraham, E.P. 1940. An enzyme from bacteria able to destroy penicillin. *Nature*. 146: 837-42.
4. Chopra, I. 2000. New drugs for superbugs. *Microbiol. Today*. 47: 4-6.
5. Daszak, P., Cunningham, A.C and Hyatt, A.D. 2000. Emerging infectious diseases of wild life, threats to biodiversity and human health. *Science*. 287: 443-49.
6. Diwan, D.P., Yashashree, A. and Gadhikar. 2012. Assessment of phytochemical composition and antibacterial activity of different extracts of *Merremia emergenata* leaves against oral microflora to improve dental hygiene. *Int J Pharm Pharm Sci.* 4(3): 621-623.
7. Hancock, E.W. 2005. Mechanisms of action of newer antibiotics for Gram-positive pathogens. *Lancet Infect Dis*. 5: 209-218.
8. Irobi, O.N., Moo-Young, M., Anderson, W.A. and Daramola, S.O. 1994. Antimicrobial activity of the bark of *Bridelia ferruginea* (Euphorbiaceae). *Int J Pharm*. 34: 87-90.
9. Neyanila, S.K., Prakash Yoganandam, G. and Gopal, V. 2013. Medicinal value of *Merremia tridentata* (L.) Hallier. f. (Convolvulaceae) – A Pharmacognostic Approach. *Intern.J. Pharm.*. 3(1): 6-40.
10. York, H., de Wet. and Van Vuuren, S. F. 2011. "Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa," *J Ethnopharmacol*. 135 (3): 696–710.

Table 1: Antibacterial activity of aerial part extracts of *M. tridentata* in different solvent extracts

Species	Methanol					Chloroform					Petroleum ether				
	250 µg/ml Z	500 µg/ml	1000 µg/ml	2000 µg/ml	G	250 µg/ml	500 µg/ml	1000 µg/ml	2000 µg/ml	G	250 µg/ml	500 µg/ml	1000 µg/ml	2000 µg/ml	G
Staphylococcus hominis	6	7	8	2	30	7	8	10	10	26	6	6	7	10	31
Bacillus subtalis	10	10	12	15	28	8	8	10	10	30	7	8	8	13	30
Staplococcus cereus	11	11	19	20	25	10	11	11	14	26	7	8	10	12	29
Escherichia . coli	7	7	8	8	30	10	16	17	20	28	7	6	6	11	27
Staphylococcus aureus	10	15	17	18	32	9	8	9	10	28	5	8	10	11	28

The values express the zone of inhibition (in mm)

Table 2: Antibacterial activity of root extracts of *M. tridentata* in methanolic and petroleum ether solvent extracts.

Species	Methanolic					Petroleum Ether				
	250 µg/ml	500 µg/ml	1000 µg/ml	2000 µg/ml	G	250 µg/ml	500 µg/ml	1000 µg/ml	2000 µg/ml	G
Staphylococcus hominis	10	10	11	16	30	6	7	8	13	32
Bacillus subtalis	8	10	12	14	28	9	10	11	19	30
Staplococcus cereus	4	10	12	13	27	6	7	7	10	30
Escherichia coli	8	8	8	10	26	7	9	9	10	29
Staphylococcus aureus	5	10	12	15	28	9	10	11	14	30

The values express the zone of inhibition (in mm)

Table 3: Antibacterial activity of flower extracts of *M. tridentata* in methanolic and chloroform solvent extracts.

Species	Methanolic					Chloroform				
	250 µg/ml	500 µg/ml	1000 µg/ml	2000 µg/ml	G	250 µg/ml	500 µg/ml	1000 µg/ml	2000 µg/ml	G
Staphylococcus hominis	6	8	10	11	30	7	8	10	11	27
Bacillus subtalis	8	10	10	17	27	9	9	10	10	30
Staplococcus cereus	7	9	10	12	28	10	12	14	15	26
Escherichia coli	6	6	8	10	30	6	7	10	11	28
Staphylococcus aureus	12	15	18	20	29	12	14	14	15	32

The values express the zone of inhibition (in mm)

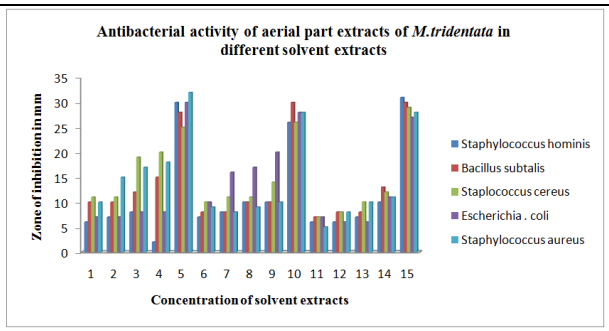

Figure-1: Antibacterial activity of aerial part extracts of *M. tridentata* in different solvent extracts

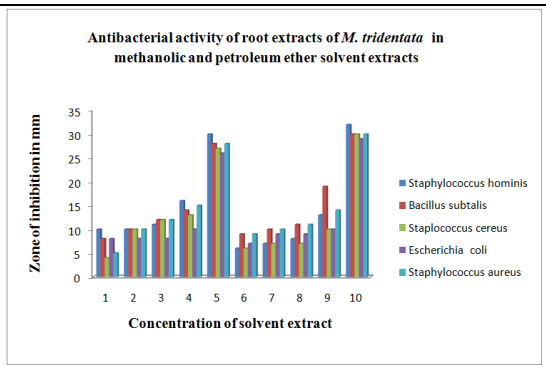

Figure-2: Antibacterial activity of root extracts of *M. tridentata* in methanolic and petroleum ether solvent extracts.

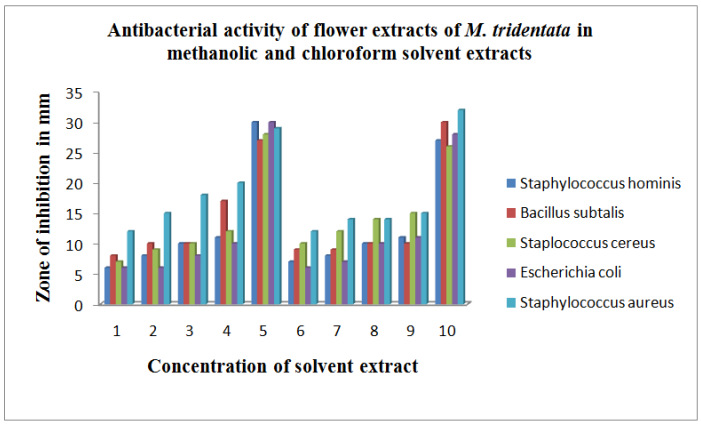

Figure 3: Antibacterial activity of flower extracts of *M. tridentata* in methanolic and chloroform solvent extracts.

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REVIEW ARTICLE

Review on Potential Role of Flavonoids for Treating Inflammatory Bowel Disease; A Special Focus on Different Targets

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ABSTRACT

Ulcerative colitis also called inflammatory bowel disease, unclear etiology affecting the rectum and colon. Ulcerative colitis patients require continuous treatment. Genetic, environmental, intestinal immune response, the intestinal microbiome and luminal factors are responsible for the cause of ulcerative colitis. Because their pathogenesis is not fully known, have produced inefficient drugs and those that have confirmed inefficacy does severe adverse effects that impair their long-term usage. Flavonoids are natural substance; they belong metabolites of plants with a polyphenolic structure, originate in medicinal plants, vegetables and fruits. Flavonoids are right health-promoting products and serve as a vital constituent in a wide variety of nutraceuticals, pharmaceuticals and therapeutic. The present study we discussed the classification and importance of the flavonoids. Flavonoids used to treat inflammatory bowel disease due to its free radical scavenging, anti-inflammatory, microbial antagonistic activity, and cyclooxygenase enzyme inhibition. Flavonoids interact virtually with the TLR4/MyD88 signallingcascades, MD-2/TLR4 complex, interleukin-6, NLRP3, interleukin-1βand interleukin-8, and (COX-2)NF-kB, PPAR/Pathway, MAPK, NLRP3. This literature reviewed the flavonoid classes evaluated in various possible mechanisms with multiple targets with significant effects.

Keywords: Ulcerative colitis, Flavonoids, antioxidant property, inflammatory bowel disease, cyclooxygenase

INTRODUCTION

UC (Ulcerative colitis) is a long-term inflammatory disorder that affects the large intestine and rectum (1).Worldwide problematic of UC continues to increase, along with the allied health care and social expenses. UC is a long-term disease with no remedial interventions, save colectomy, treatment is maximum regularly lifetime. Different factors, genetic, environmental, luminal factors and immune dys regulations indicated to enhance ulcerative colitis (2,3). UC is the regularly occurred forms of IBD(Inflammatory bowel disease) and has been around since 1859. UC is described by oral mucosa inflammation that initiates in the rectum and spreads proximally in the colon in a linear pattern (4).UC mechanism is complex and is not fully understood. Even so, the data presently available allows the development of an established working model consisting of multiple contributing factors and frame works.UC may be driven initially by structural intestinal dysfunction that causes an inflammatory disorder known as Crohn's disease. Alternatively, barrier damaged by inflammatory mediators, a cell in the lamina propria, leads to the disease's chronicity (5, 6).

Phytomedicine is attracting extra importance among UC infected patients. Fifty percent of UC infected patients have preferred complementary and alternative therapies, including herbal treatment. Herbal medicine in UC infected persons connected with low acceptability and apparent incompetence of modern therapy. Therefore, it is essential to be aware of the presently available phytobioactive constituents and their action. (7, 8).

Flavonoids are natural products; they belong primarily to secondary metabolites of plants through a phenolic compound, commonly present in medicinal plants, green leaves and fruits. Flavonoids have countless therapeutic effects allied with multiple illnesses such as malignancy, Alzheimer's disease, heart diseases, antioxidant and antimicrobial etc. (9, 10). Phytochemicals (flavonoids) are responsible for a wide-ranging of health-promoting products and serve as a multivariable substance in a wide range of nutraceuticals, pharmaceuticals, therapeutic and beautifying effects. Their free radical scavenging, antimicrobial, anticarcinogenic, and inflammation inhibitory can modulate primary enzyme functions inside the cells. Flavonoid compounds are also responsible for inhibiting numerous cellular enzymes, such as phosphoinositide 3-kinase,xanthine oxidase, cyclooxygenase and lipoxygenase (11, 12).In plants naturally, flavonoid phytoconstituents are low molecular weight phenolic compounds present in various medicinal plants.

Flavonoids represent one of the most influential groups of natural products, and it includes over 9,000 chemicals. The term 'flavonoid' is implemented to molecules structurally propylbenzene derivatives with C15, C16 skeleton molecules(13, 14). Flavonoids have a wide range of beneficial activities in animals, plants, and microorganisms. Flavonoids have historically recognised to produce in exact locations in plants. Flavonoids are accountable for the colour of floras and fruit, spreading to assist in germination of seeds and growth development (15). The current review discusses the recent advances of exploration and progress on flavonoids and to their uses as ulcerative colitis.

Classification of Flavonoids

Flavonoids classified several subgroups based on carbon on C and B ring. Those flavonoids B ring connected in location 3 of the C ring is called isoflavones. Those with the 4th ring in place 4 are neoflavanoids, althoughthe 2nd ring in place two further divided into some subcategories based on the C ring structure. Flavanols, catechins, flavone, flavanones, anthocyanins, isoflavones and chalcones (Table 1)(16).

Flavanols

Flavonols are phenolics comes under the family of flavonoids. Flavanols were having subcategory of flavonoids which consist of is or hamnetin and ampelopsin. They are colourless compounds found in leaf cabbage, germination seeds, onions, allium and beans. Quercetin is an antiallergic that helps control high temperature and allergic

reactions, and It also has anti-inflammatory properties. Myricetin, Isorhamnetin and other flavanols accompany potent anti-inflammatory, and free radical scavenging activities cause to prevent chronic disease (17).

Catechins

Catechins are phenol functional group substance first extracted from the *Acacia catechu*. Phytochemical catechins are mainly present in white and greente as. Researchers believe catechins may help with chronic fatigue syndrome symptoms and treat heart and neurological health conditions (18). Catechins caused to reduce the very low-density and low-density lipoproteins (bad cholesterol), which leads to preventing atherosclerosis.

Flavones

Flavones belong to a subgroup of flavonoids widely spread in plants. Flavones synthesised by various pathways based on they possess hydroxylated B-ring and C- or O-glycosylation. Flavones are significant health benefits as essential natural products associated with plant signalling and protection andcrucial human nutrition elements (19).

Flavanones

Flavanones include eriodyctiol, naringenin and hesperidin. Citrus fruits abundantly found flavanones. Flavanones manage heart-related (cardiovascular), antimutagenic, free radical scavenging and anti-inflammatory activity (20).

Anthocyanins

Anthocyanins are aqueous soluble coloured pigments that belong to the phenolic compounds. The anthocyanin pigments are glycosylated forms. The anthocyanins are accountable for the colours (red, purple, and blue) in vegetables and fruits. There are high levels of anthocyanin in berries, oranges, and some tropical fruits. These are bioactive substances as nutraceuticals. It has commonly used to treat many other disorders, appetite stimulant, choleretic agent, and the colour pigments are pharmaceutical ingredients. Anthocyanins are the main components in maintaining better health and disease prevention (21).

Isoflavones

The subgroup contains apigenin, wogonin, luteolin, diosmin, baicalein. Isoflavones are incredibly abundant in legumes and soy products. They are substance regulate estrogens, which means they are molecules that behave like hormonal estrogens. Researchers believe that controlling the defects of hormonal cancers, such as prostate, breast, and endometrial, could be beneficial, although the study findings currently mixed. Isoflavones having good free radical scavenging activity, but their action on malignance is uncertain. Isoflavones also tested as a way to relieve the effects of menopause (22).

Role of flavonoids in Inflammatory bowel disease

Pharmacological properties to flavonoids, enzyme activity inhibition, antioxidant properties, and suppressive inflammation, reduce the severity of various inflammatory diseases, including IBD (Figure 1) (23). The possible beneficial effects of flavonoids on intestinal inflammation observed by Galsanov et al. 1976.

Influences of flavonoids in eicosanoid metabolism

Eicosanoids (prostaglandins, thromboxane, leukotrienes etc.)are biological molecules derived from arachidonic acid metabolism and show an essential role in inflammatory bowel disease (24). Earlier research studies reported enhancing eicosanoid concentrations in inflammation tissue areas compared to normal mucosa in human inflammatory bowel disease. The increased level of enzymes involved in the breakdown of eicosanoids has linked with inflammatory conditions. Flavonoids impair COX-1, COX-2, PGE2 resultant stop the inflammatory process (25, 26).

Immunoregulatory characteristics for flavonoids

The injection of flavonoids as rutin, Naringenin, Hesperidin, and quercitrin in the sodium dextran sulfate method significantly reduced the augmented concentration of the various cytokines assessed in the impaired colon. Incubation of RAW 264.7, LPS-activated macrophages with flavonoid (quercetin, baicalin) cells reduced IL-1 and TNFα. Rutin significantly reduced increased IL-1 levels (27, 28).

Quercetin has anticancer properties by decreasing interferon-gamma, and tumour necrosis factor-alpha development in concanavalin A triggered isolated lymphocytes-T. Incubation of colorectal adenocarcinoma cells, cytokine-stimulated epithelial cells and colon cancer cells (HT-29) with flavonoids like genistein pointedlydecrease IL-8 excretion in cell culture(29, 30, 31, 32).The mitogen-activated protein kinase signalling pathway enhances instant initial gene activation and transcription of cellular reactions such as migration, cytokine synthesis, programmed cell death. The mitogen-activated protein kinase elements are signalling mechanism consist of a three-tiered kinase cascade consisting of mitogen-activated protein kinase. The flavonoids are interfering the mitogen-activated protein kinase (33, 34).

The antioxidant property of flavonoids

Human inflammatory bowel disease has related to significant oxidative stress, excessive active nitrogen and oxygen species reactive nitrogen species generation in the intestinal tissue (35).Production of reactive species induced DNA damage, lipid peroxidation, protein modification, apoptosis (36, 37). Flavonoids have good antioxidant properties which lead to decrease the excessive generated reactive species. Neutralise the reactive radical protect the changes of DNA, lipid peroxidation, protein modification etc.

Impact of flavonoids on the function of the intestinal barrier

In vitro experiments have confirmed that flavonoidsas daidzenin, naringenin, and morin augment the epithelial barrier's role. (38). Flavanone incubation with intestinal colon carcinoma epithelial cells leads to electrical resistance across the cells' monolayers, which corresponds to tight junction integrity development (39).Intestinal junction integrity observed by blotting and microscopic method. Incubation of flavonoids enhances the cytoskeletal assembly of tight junction proteins. Unintended pathways may also contribute to flavonoids' positive effects in sustaining the gastrointestinal tract activity if they directly impact tight junction protein function. Proinflammatory cytokines, such as TNF-α and IL-6 have been reported to damage the epithelial barrier role through apoptosis mechanisms (40, 41). As a result, these compounds' inhibitory action on TNF-α and IL-6 expression can also enhance the intestinal absorption noted in experimental colitis (42).

Interaction of flavonoids with gut microbiome

Several researchers have described that nutrition contains active molecules, such as flavonoids, may be considered supplementary medicines for inflammatory bowel disease due to their antimicrobials. Flavonoids on intestinal microbiome composition may also pay the beneficial effects on intestinal inflammation (43, 44). Naringenin has reported inhibiting multiplication and adherence of *Salmonella typhimurium* pathogens, on colon cancer cells and flavanone, which has increased the spread and adherence of probiotic note *L.rhamnose*; this probiotic has described as having beneficial effects on human intestinal inflammation (45).

Anti-inflammatory mechanism of flavonoids

Systematic research suggested, and results showed that flavonoids might affect critical pathways, such as metabolism, processing of genetic information, processing of environmental data, cellular processes, organism systems, and frameworks associated with human diseases (Table 2). Enhancement of pathways linked to diseases demonstrated the multidimensional medical benefits of flavonoids that could impact human diseases such as IBD (46).

CONCLUSION

The numerous experiments conducted with flavonoids effort on their intestinal anti-inflammatory activity - the results are considered therapies for inflammatory bowel disease. In research studies, flavonoids results showed usefulness, and their mechanism of action is equal to those mentioned in medications commonly used in treatment. Flavonoids consumption of vegetables gives them a healthy practice. The clinical studies need to validate their exact fundamental role in treating these inflammatory bowel disorders. The majority of human studies consider flavonoid-containing plants and have shown that they can induce clinical response in inflammatory bowel disease patients. In detail, clinical studies (*in-vivo*) explain the various flavonoids' effectiveness and security in these IBD and consider them healthy, beneficial approaches.

Conflict of Interest: Nil
Authors state that there is no conflict of interest.

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Abbreviations
UC: Ulcerative colitis, IBD: Inflammatory bowel disease, LPS: Lipopolysaccharide, STAT Signal transducer and activator of transcription, DSS: Dextran sulphate sodium,IL-6 Interleukin-6,TNF-α: Tumour necrosis factor α,NF-Kβ: Nuclear factor kβ,COX: Cyclooxygenase, TGF: Transforming growth factor, MAPK: Mitogen-activated protein kinase, TLR4: Toll-like receptor, APC: Antigen-presenting cells, PPAR-γ:Peroxisome proliferator-activated receptor-gamma.PXR: Pregnane X receptor.

REFERENCES

1. KobayashiT, SiegmundB, Berre LC, *et al.* Ulcerative colitis. Nat Rev Dis Primers2020; 6: 74.
2. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel J-F. Ulcerative colitis. The Lancet2017;389(10080):1756-1770.
3. Matsuoka K, Kobayashi T, Ueno F, *et al.* Evidence-based clinical practice guidelines for inflammatory bowel disease. J. Gastroenterol 2018;53(3):305-353.
4. Magro F, Gionchetti P, Eliakim R, *et al*. Third european evidence-based consensus on diagnosis and management of ulcerative colitis. part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. JCrohns Colitis2017;11(6):649-670.
5. Ungaro R, Colombel J-F, Lissoos T, Peyrin-Biroulet L. A treat-to-target update in ulcerative colitis. Am JGastroenterol 2019;114(6):874-883.
6. Colombel J-F, D'Haens G, Lee W-J, Petersson J, Panaccione R. Outcomes and strategies to support a treat-to-target approach in inflammatory bowel disease: A systematic review. JCrohns Colitis2020;14(2):254-266.
7. Holleran G, Scaldaferri F, Gasbarrini A, Currò D. Herbal medicinal products for inflammatory bowel disease: A focus on those assessed in double-blind randomised controlled trials. Phytother Res2020;34(1):77-93.
8. Kennedy J, Wang C-C, Wu C-H. Patient disclosure about herb and supplement use among adults in the us. EvidBased Complementary Altern Med. 2008;5(4):451-456.
9. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. FoodNutr Res. 2017;61(1):1361779).
10. Lee YK, Yuk DY, Lee JW, *et al*. (-)-Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of beta-amyloid generation and memory deficiency. Brain Res 2009; 1250:164-174.

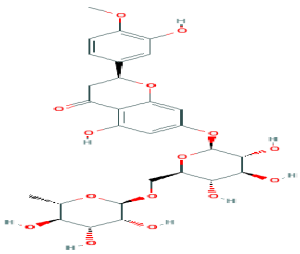
11. Iio M, Moriyama A, Matsumoto Y, Takaki N, Fukumoto M. Inhibition of xanthine oxidase by flavonoids. Agric Biol Chem1985;49(7):2173-2176.
12. Walker EH, Pacold ME, Perisic O*et al*. Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. Mol Cell 2000;6(4):909-919.
13. Yonekura-Sakakibara K, Higashi Y, Nakabayashi R. The origin and evolution of plant flavonoid metabolism. Front Plant Sci 2019; 10:943.
14. Rauter, A. P, Ennis, M, HellwichK. H, Herold BJ, Horton D, Moss GP, *et al*. Nomenclature of flavonoids (IUPAC recommendations 2017). Pure Appl Chem2018;90: 1429–1486.
15. Griesbach R. Biochemistry and genetics of flower colour. Plant Breed Rev2005; 25: 89–114.
16. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *J Nutr Sci*2016;5: e47.
17. Martínez-Lüscher J, Brillante L, Kurtural SK. flavonol profile is a reliable indicator to assess canopy architecture and the exposure of red wine grapes to solar radiation. Front Plant Sci2019;10.
18. Jaramillo Flores ME. Cocoa Flavanols: Natural agents with attenuating effects on metabolic syndrome risk factors. Nutrients2019;11(4):751.
19. Jiang N, Doseff AI, Grotewold E. Flavones: From Biosynthesis to Health Benefits. PlANTS-BASEL2016;5(2):27).
20. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr2004;79(5):727-747.
21. Iwashina T. Flavonoid properties in plant families synthesizing betalain pigments. *Nat* Prod Commun 2015;10(6):1103-1114.
22. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res 2017;61(1):1361779.
23. Szkudelska K, Nogowski L. Genistein--a dietary compound inducing hormonal and metabolic changes. J Steroid Biochem Mol Biol2007;105(1-5):37-45.
24. Vezza T, Rodríguez-Nogales A, Algieri F, Utrilla MP, Rodriguez-Cabezas ME, Galvez J. Flavonoids in inflammatory bowel disease: A Review. *Nutrients*2016;8(4):211.
25. Kaufmann HJ, Taubin HL. Nonsteroidal anti-inflammatory drugs activate quiescent inflammatory bowel disease. Ann Intern Med1987;107(4):513-516.
26. Surh YJ, Chun KS, Cha HH, *et al*. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutat Res2001;480-481:243-268.
27. Dubois RN, Abramson SB, Crofford L, *et al*. Cyclooxygenase in biology and disease. FASEB J1998;12(12):1063-1073.
28. Dou W, Zhang J, Zhang E, *et al*. chrysin ameliorates chemically induced colitis in the mouse through modulation of a PXR/NF-κBsignaling pathway. J Pharmacol Exp Ther2013;345(3):473-482.
29. Brückner M, Westphal S, Domschke W, Kucharzik T, Lügering A. Green tea polyphenol epigallocatechin-3-gallate shows therapeutic antioxidative effects in a murine model of colitis. J Crohns Colitis2012;6(2):226-235.
30. Mascaraque C, Aranda C, Ocón B, *et al*. Rutin has intestinal antiinflammatory effects in the CD4+ CD62L+ T cell transfer model of colitis. Pharmacol Res2014; 90:48-57.
31. Comalada M, Camuesco D, Sierra S, *et al*. In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. Eur J Immunol2005;35(2):584-592.
32. Cui L, Feng L, Zhang ZH, Jia XB. The anti-inflammation effect of baicalin on experimental colitis through inhibiting TLR4/NF-κB pathway activation. Int Immunopharmacol2014;23(1):294-303.
33. Melgarejo E, Medina MA, Sánchez-Jiménez F, Urdiales JL. Targeting of histamine producing cells by EGCG: a green dart against inflammation?. J PhysiolBiochem2010;66(3):265-270.
34. Jeffrey KL, Camps M, Rommel C, Mackay CR. Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. Nat Rev Drug Discov2007;6(5):391-403.
35. Broom OJ, Massoumi R, Sjölander A. Alpha2beta1 integrin signalling enhances cyclooxygenase-2 expression in intestinal epithelial cells. J Cell Physiol 2006;209(3):950-958.

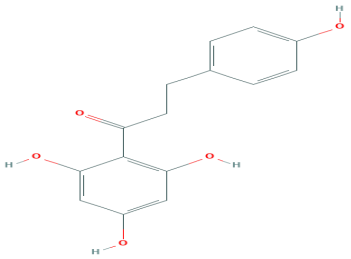
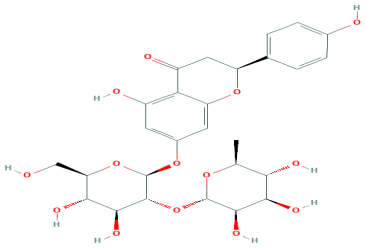
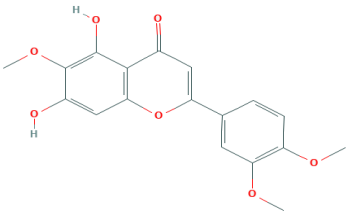
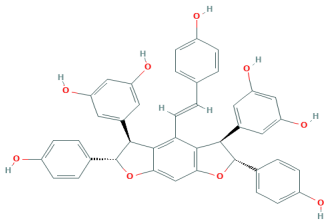
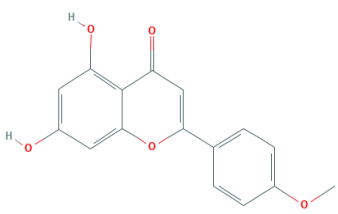
36. Rezaie A, Parker RD, Abdollahi M. Oxidative stress and pathogenesis of inflammatory bowel disease: an epiphenomenon or the cause?. Dig Dis Sci 2007;52(9):2015-2021.
37. Achitei D, Ciobica A, Balan G, Gologan E, Stanciu C, Stefanescu G. Different profile of peripheral antioxidant enzymes and lipid peroxidation in active and non-active inflammatory bowel disease patients. Dig Dis Sci2013;58(5):1244-1249.
38. Piechota-Polanczyk A, Fichna J. Review article: the role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. NaunynSchmiedebergs Arch Pharmacol2014;387(7):605-620.
39. Geier MS. Butler RN.; Howarth, G.S. Inflammatory bowel disease: Current insights into pathogenesis and new therapeutic options; probiotics, prebiotics and synbiotics. Int. J. Food Microbiol2007; 115: 1–11.
40. Noda S, Tanabe S, Suzuki T. Differential effects of flavonoids on barrier integrity in human intestinal Caco-2 cells. J Agric Food Chem 2012;60(18):4628-4633.
41. Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. J Biol Chem 2011;286(36):31263-31271.
42. Bruewer M, Luegering A, Kucharzik T, *et al*. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. J Immunol2003;171(11):6164-6172.
43. Azuma T, Shigeshiro M, Kodama M, Tanabe S, Suzuki T. Supplemental naringenin prevents intestinal barrier defects and inflammation in colitic mice. J Nutr2013;143(6):827-834.
44. Bellaguarda E, Chang EB. IBD and the gut microbiota--from bench to personalised medicine. Curr Gastroenterol Rep2015;17(4):15.
45. Swidsinski A, Weber J, Loening-Baucke V, Hale LP, Lochs H. Spatial organisation and composition of the mucosal flora in patients with inflammatory bowel disease. J Clin Microbiol 2005;43(7):3380-3389.
46. Larrosa M, Luceri C, Vivoli E, et al. Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. Mol Nutr Food Res2009;53(8):1044-1054.
47. Qiu T, Wu D, Yang L, et al. Exploring the Mechanism of Flavonoids Through Systematic Bioinformatics Analysis. Front Pharmacol2018; 9:918.

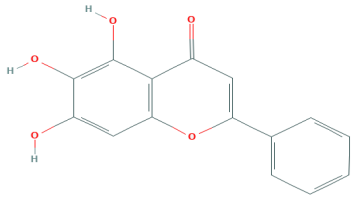
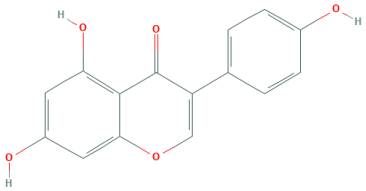
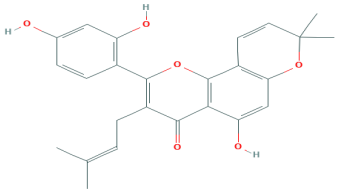
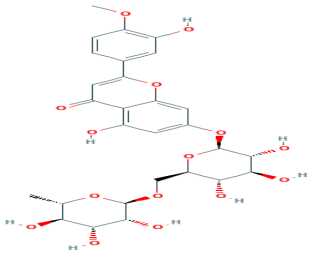
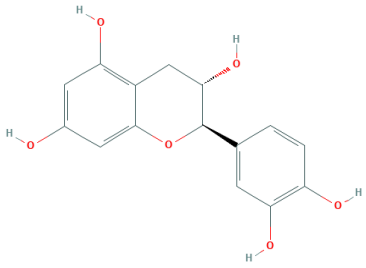
Table 1: Classification of Flavonoids

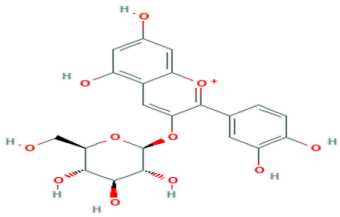
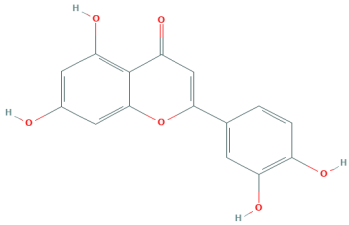
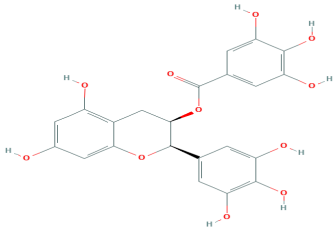
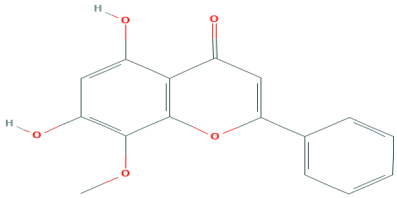
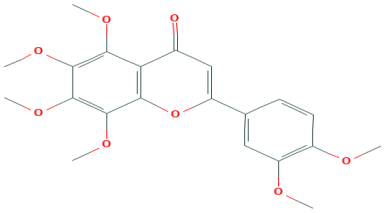
Classification of Flavonoids	
Flavanols	Quercetin, Myricetin, Kaempferol, Isorhamnetin, Ampelopsin
Catechins	Epicatechin, Epigallocatechin-3-gallate, Catechin
Flavone	Apigenin, Wogonin, Luteolin, Diosmin, Morusin, Baicalein, Acacetin, Eupatilin
Flavanones	Naringenin, Hesperidin
Anthocyanins	Cyanidin-3-o-glycoside
Isoflavones	Genistein
Chalcones	Phloretin

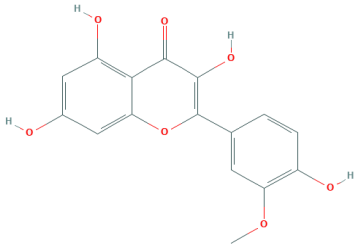
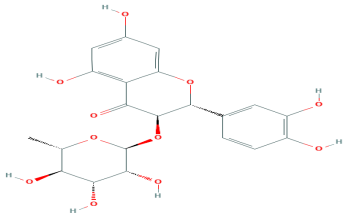
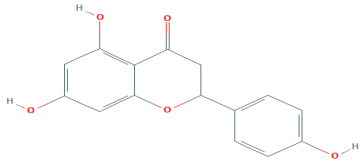
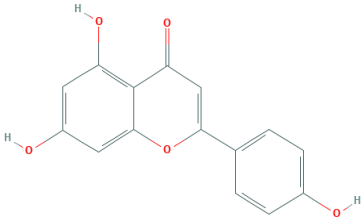
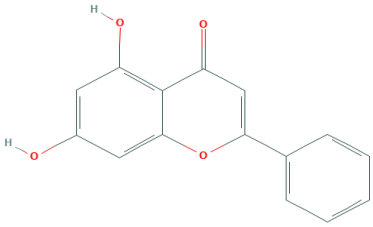
Table 2. Intestinal anti-inflammatory activity of flavonoids

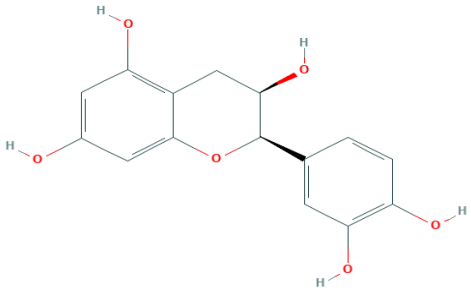
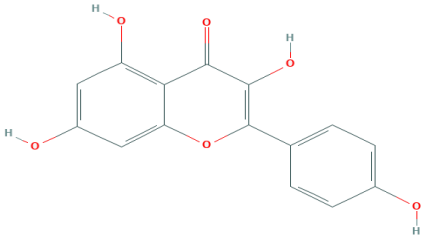
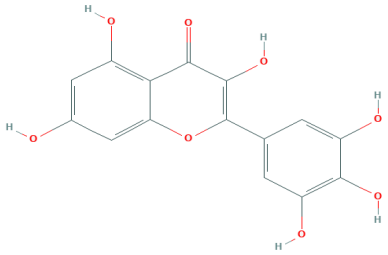
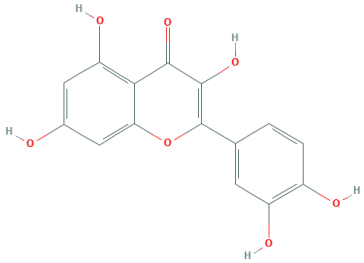
S.NO	Names and Structures	Targets	Model	Reference
1	**Hesperidin** 	TNF-α, NrF2, SOD, GSH	DSS-induced colitis and caco-2 cells	Guo K *et al*., 2019

2	**Phloretin** 	NF-Kb, TLR4, PPAR γ pathway, NLRP3	DSS -induced colitis	Zhang Z *et al.*, 2018
3	**Naringin** 	NF-kB, Pathway to PPAR, MAPK, NLRP3	DSS- induced colitis	Cao H *et al*.,2018
4	**Eupatilin** 	TNF-α	DSS-induced colitis	Zhoua K *et al.*, 2018
5	**Ampelopsin** 	IRAK1/TRAF6/ NF-KBsignalling pathway	DSS-induced colitis	Chen YL *et al.*, 2018
6	**Acacetin** 	NF-KB (p50), COX-2, PGE2	IL-1β-induced caco-2 cells	Prasad N and Yadav U.C.S., 2018

7	**Baicalein** 	NF-KB and MAPK pathway	*E.coli* induced colitis	Liang S*et al*.,2019
8	**Genistein** 	TLR4/ NF-KB signal	DSS induced colitis	Zhang R *et al*., 2017
9	**Morusin** 	SOD, CAT, IL-1β, TGF-β1, MMP2 and MMP9 activities	TNBS-induced colitis	Vochyanova Z *et al*.,2017
10	**Diosmin** 	TNF-α, COX-2, MPO, MDA, GSH and caspase-3 expression	Acetic acid-induced colitis	ShalkamiA S *et al*.,2018
11	**Catechin** 	NF-kB, MAPKs, NrF2, GSH, GPO, STAT1/3	DSS-induced colitis	Fan F *et al*.,2017

12	**Cyanidin-3-O-Glycoside** 	NF-kB, TNF-α, IL-6, COX-2, NrF2	CaCo2 cells induced colitis	Ferai D *et al.*,2016
13	**Luteolin** 	TNF-α, IL-6, MDA, NrF2, iNOS	DSS-induced colitis	Li Y *et al.*, 2016
14	**Epigallocatechin-3-Gallate** 	COX-2 mRNA	TNBS induced colitis	Lin Y Z*et al.*, 2007
15	**Wogonin** 	TLR4-MyD88-TAK1-mediated NF-κB pathway	LPS induced Caco-2 cells	Wang W *et al.*, 2015
16	**Nobiletin** 	Akt-NF-κB-MLCK pathway, COX-2 and iNOS expression,	TNBS induced colitis	Xiong Y *et al.*,2015

17	**Isorhamnetin** 	PXR	DSS induced colitis	Dou W, *et al.*,2014
18	**Astilbin** 	TGF-β, IL-10	DSS-Induced colitis	Ding Y *et al.*, 2014
19	**Naringenin** 	TLR4/NF-κB signalling pathway	DSS-Induced colitis	Duo W *et al.*, 2013
20	**Apigenin** 	STAT3, NF-κB signalling, MPO, COX-2	DSS-Induced colitis	Xiao-Yu Ai*et al.*, 2013
21	**Chrysin** 	PXR/NF-κB Signaling pathway	TNBS and DSS colitis induced	Duo W *et al.*, 2013

22	**Epicatechin** 	COX-2	TNBS -induced colitis	Vasconcelos P *et al.*,2012
23	**Kaempferol** 	MPO, PGE(2) TFF3 mRNA	DSS-Induced colitis	Park MY *et al.*, 2012
24	**Myricetin** 	NF-Kb DNA binding activity (IL 12)	LPS induced colitis	Kang B Y*et al.*,2005
25	**Quercetin** 	TNF-α-dependent NF-κB activation	TNBS-induced colitis	Heejung*et al.*, 2005

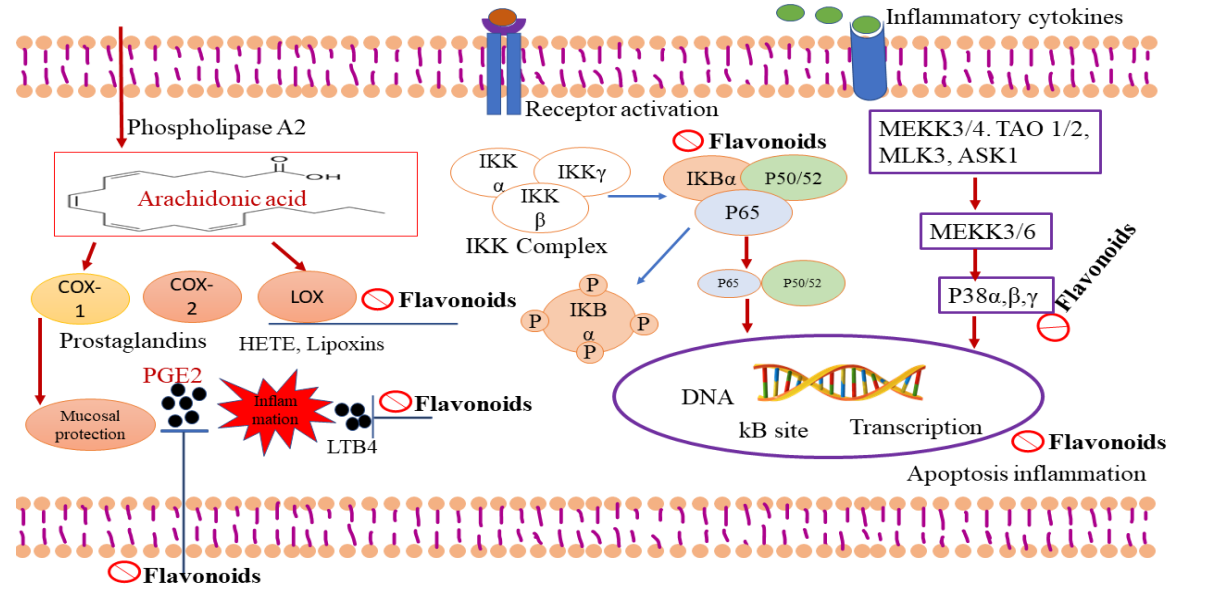


Figure 1. Pharmacological role of flavonoids in the management of Inflammatory bowel disease

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REVIEW ARTICLE

Phytochemical and Pharmacological Perspectives of *Memecylon randerianum* S.M. Almeida & M.R. Almeida: A Mini Review

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ABSTRACT

The majority of plants around us show vital role in the traditional and modern medicinal systems. They have been consumed by most of the people around the world since ancient period. The pharmacological properties and appropriate formulations of medicinal plants has to be disseminated for the effective utilization of their bioproducts in the health care sector. *Memecylon randerianum* S.M. Almeida & M.R. Almeida is an indigenous species of the family Melastomataceae, utilized in traditional medicine for the treatment of bacterial infections, diabetes, inflammatory and skin disorders such as herpes, chickenpox, and psoriasis. The distribution of the plant is reported from evergreen, semi-evergreen and sacred groves of Southern Western Ghats. The literature survey described that *Memecylon randerianum* is rich in valuable secondary metabolites like flavonoids, glycosides, phenols, resins, saponins, tannins and some acidic compounds. Only one novel compound, namely memecyclaene has been identified and isolated in this species. The leaf extract of this plant has displayed potential pharmacological activities such as antimicrobial, antidiabetic, anti-inflammatory, antioxidant properties. This review gives comprehensive information for future research on the pure bioactive compounds and its therapeutic potential of the plant. Further investigations on these aspects will certainly assist to derive an effective pharmaceutical drug through advanced *in vitro* and *in vivo* studies.

Keywords: Memecylaene, *Memecylon randerianum*, Pharmaceutical drug, Pharmacological activities, Secondary metabolites.

INTRODUCTION

Western Ghats is one of the most beautiful biodiversity hotspots in the world. The richness of Western Ghats is increased by the exclusive varieties of medicinal plants [1]. Many medicinal plants including endemic and endangered species are well conserved and protected in this region. The medicinal plants are major source for the

preparation of many ethno medicinal formulations since ancient times. The traditional knowledge about numerous medicinal plants are well documented and widely practised for the treatment of various disorders. The existing knowledge on the ethnobotanicals directs various research investigations to the effective drug discovery and its development [2]. The traditional use of each plant should be validated through effective research studies on phytochemical and pharmacological aspects. The nutraceutical, pharmaceutical and cosmeceutical industries are constantly focused on these perspectives for the effective use and commercialization of bioproducts.

The seventh largest family, Melastomataceae was less studied from a phytochemical and pharmacological view [3]. Many members of this family are very familiar for practice in ethnomedicine as antioxidant, antihypertensive, antihyperglycemic, hemostatic, antihepatitis, and antidiarrhoic drugs [4]. The isolation of phytochemicals such as terpenoids, steroids, simple phenolics, flavonoids and a vast range of polyphenols was reported from this family [5]. The other economic importance of this family includes timber shrubs, edible fruit plants, dye plants, and several ornamental cultivars [6].The genus memecylon belongs to the Family melastomataceae consists of more than 300 species that are widely occurs throughout in the Western Ghats of India. They occupy diverse forest habitats in the Old World tropics and shows high regional endemism [7]. Memecylon is characterised by small to medium-sized trees with very hard wood, having axillary inflorescences of simple or paniculate cymes or umbels, tetramerous flowers, eight isomorphic stamens, anthers dehiscing by slits, large connectives and 1-seeded berries crowned with the calyx margin [8]. They are rich source of medicinally important constituents such as flavonoids, tannins, terpenoids, alkaloids, phenols etc. It is well documented that several Memecylon species have been reported to be used by tribals in the treatment of skin disorders, stomach disorders, herpes, chickenpox, leucorrhoea, polyuria, menorrhagia, dysentery and also in the treatment of bacterial infections and inflammation in Ayurveda and Siddha [9]. In Kerala, only a few studies were reported on the potential phytochemicals and pharmacological importance of *Memecylon randerianum*.

Memecylon randerianum S.M.Almeida & M.R.Almeida belongs to the Family Melastomataceae is an indigenous medicinal plant widely distributed in evergreen and semi-evergreen forests of Western Ghats. The occurrence of the plant is also reported from some of the sacred groves of Kerala. This plant is a large woody shrub that grows up to 5-6 m tall. The status of this plant is described as occasional. The plant is known by various names like Kaikkathetti, Kashara, Kazhavu, Koovachekki (Malayalam) Malamthetti, Perungaca, Vacci (Tamil) Doddanekkare (Kannada) and Ollekodi (Tulu) and shows resemblance with Ixoracoccinea. Flowering and fruiting of the plant is predominantly observed during the summer season (February to May). This plant is used in ethno medicine for the control of diarrheal, bacterial infections, inflammatory and skin disorders including herpes and chickenpox. In the present review, the phytochemical and pharmacological research works on *Memecylon randerianum* were described. A thorough literature study was conducted for research articles related to botanical profile, traditional uses, phytochemistry and pharmacological analysis on *Memecylon randerianum*. Both *in vitro* and *in vivo* investigations on *M. randerianum* was included in this review.

Botanical Profile of the plant

The name, *Memecylon randerianum* S.M.Almeida & M.R.Almeida was first published in Journal of the Bombay Natural History in 1989. The synonyms of this plant are *Memecylon malabaricum* (Clarke) Cogn. and *Memecylon amplexicaule* Roxb. var. malabarica Clarke in Hook. f. The phylogenetic analysis conducted by Ramasetty *et al*., [10] clearly distinguished *Memecylon malabaricum* from *Memecylon wightii* in accordance with morphological keys. Five Memecylon species were distinctly separated into three different clades in the RAPD and ISSR analysis. *M. malabaricum* and M. wightii grouped together and *M. umbellatum*, *M. edule* and *Memecylon talbotianum* grouped in the same clade with high Jaccard dissimilarity coefficient and bootstrap indicating that these grouped species are phylogenetically similar.

Taxonomic Hierarchy of the plant

Kingdom: Plantae

Division: Magnoliophyta/ Tracheophyta
Class: Magnoliatae/ Magnoliopsida
Order: Myrtales
Family: Melastomataceae

Botanical Description

It is a large woody shrub that grows up to 5 m tall. The leaves are simple, opposite, apex gradually acute and sessile. The flowers are cauliflorous and fascicled in very dense many-flowered cymes and the peduncles have 1-2 mm long pedicels short. Flowers are purplish blue in colour. Calyx tube is campanulate and truncate at apex. Petals are obovate and purplish blue in colour. Style is subulate. The berries are globose in nature. The colour of the fruits changes colour from green to red and then dark purplish as ripen. The flowering and fruiting of the plant is predominantly observed during the summer season (February to May).

Geographical Distribution of the Plant

The plants are widely seen in the Western Ghats, Evergreen Forests and semi-evergreen forests, and also in sacred groves, but it is endemic to Southern Western Ghats. The other countries and its locations are noted below;
Maharashtra: Sindhudurg
Kerala: Idukki, Kollam, Kozhikode, Malapuram, Pathanamthitta, Thiruvananthapuram, Wynad, Tamil Nadu: Coimbatore, Dindigul, Kanniyakumari, Madurai, Nilgiri.
The occurrence of the plant is also reported from some of the sacred groves of Kerala. Mandan Jyothi[11] studied the diversity parameters such as relative density, relative frequency, relative basal area and Important Value Index (IVI) of different plants in the sacred groves of Northern Kerala and recorded the diversity characteristics of *M.randerianum* from MadayiKavu located at Kannur district.

Traditional Uses

This plant has been traditionally used singly and also consumed in combination with other medicinal plants for the control of diarrhea, diabetics, bacterial infections, inflammatory and skin disorders, viral infections including herpes and chickenpox mainly in different tribes of Kerala, Karnataka and Tamil Nadu. The leaves are used in the treatment of psoriasis [12]. The roots of the plant act as ecbolic [13,14]. The wood can be used as good fuel because of its hardness. Sanilkumar and Geethukrishna [15] reported this plant in the ethnomedicinal documentation which was conducted along the coastline sacred groves of central Kerala, Southwest India. Iyengar *et al*., [16] documented the folkloric uses of this plant through personal observation and on consultation with large numbers of local folks, local popular healers, ayurvedic physicians with some interest and experiences. Mohammed and Shetty [17] made survey on dermatological ailments in coastal region of Karnataka called Udupi and they reported the use of leaves against Herpes disease from traditional healers. The leaf paste can be applied externally and decoction is used internally for this disease.

Physico-chemical parametres

The pharmacognostic evaluation gives valuable preliminary data regarding physico-chemical and elemental characteristics of various plant extracts. Asha *et al*., [18] carried out studies on physico-chemical parameters and elemental composition of the five species of Memecylon. *M. malabaricum* recorded highest values of crude protein, sodium, potassium in their investigation (Table 1 and 2).

Bioactive Phytochemicals of the plant

The previous phytochemical studies were mainly concentrated on the leaves of *Memecylon randerianum*. The metabolites such as carbohydrates, phenols, cardiac glycosides, steroids, phytosterols, saponins, terpenoids, flavonoids, tannins, alkaloids and coumarins were detected in leaves through qualitative phytochemical analysis [19]. The isolation of a novel molecule namely Memecylaene from the leaves of the plant and its anti-inflammatory

property were reported in a previous work conducted by Rekha *et al*., [20]. Twenty three phytocomponents were identified from methhanolic extract of the leaves through GC-MS analysis [21]. Palmitic acid vinyl ester (25.01%), 3-methyl octadecane (12.46%), 2,6,6-trimethyl bicycle [3.1.1] heptane(9.57%), (Z)-1,1-dimethoxy-9-octadecene (6.09%) were detected in high amount from their study. Majority of the compounds found out from their study are coming under fatty acid category. Naturally occuring fatty acid esters have a smoothing and softening effect on the skin. This could be the reason for the utilization of the plant for skin irritations. The phytochemicals and some medicinal properties of the plant were demonstrated in Figure 1.

Pharmacological Potential of the plant

The previous studies carried out in this plant have reported several medicinal properties and the responsible bioactive phytochemicals of the plant.

Anti-Fungal Activity

A number of antibiotics are used for the treatment of various bacterial infections. It always lead to many health issues. Majority of the community use many medicinal plants to treat a large number of diseases including bacterial and viral infections since ancient times [22]. The crude extract of the plants can be used in disc diffusion and broth dilution assays to test for antifungal as well as antibacterial properties [23]. Most of the memecylon species show significant antibacterial property against both gram positive and gram negative bacteria. Hegde and Hungund [24] suggested that the methanolic extract *M.randerianum* can be used for the treatment of skin infections as in the traditional knowledge since it has significant antimicrobial activity against both gram-negative *E.coli* and gram-positive *S.aureus*. They calculated the minimum inhibitory concentration of the extract by agar well diffusion and microdilution method. Sekhar *et al*., [25] made studies on the antibacterial potential of the leaves of three different memecylon species and found that *M. malabaricum* showed most effective inhibition on *Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia coli*. The methanolic extract of *M. umbellatum* and *M. malabaricum* was most potential antibacterial agent for *Staphylococcus aureus*, *Bacillus subtillis*, *Salmonella typhi* and *Escherichia coli* among other memecylon species like *M. wightii* and *M. edule*[26]. Sivu *et al*., [27] evaluated antibacterial activity against three gram positive and six gram negative bacteria in different species of Memecylon. *M. malabaricum* leaves showed strong to moderate inhibitory action on the bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Salmonella typhi* with MIC of 3.125–6.250 mg/ml [28].

Anti-Fungal Activity

Only a few antifungal studies were carried out in this species. Hullatti and Rai [28] carried out antifungal studies against seven fungal strains; *Aspergillus niger*, *A. flavus*, *A. versicolor*, *A.columnuris*, *A. tamari*, *A. flaviceps* and *Fusarium oxysporum*. The methanolic leaf extract of *M. malabaricum* showed strong inhibition on all the tested fungi and they observed maximum activity against *Aspergillus tamari* and minimum against *Fusarium oxysporum*. But the leaves show least effect against *Peridiopsoramori* [29].

Anti-oxidant Activity

The previous studies demonstrated that the leaves of *M.randerianum* have strong antioxidant properties. The extensive literatures of antioxidant studies on this plant clearly described the potential of leaf extract to scavenge reactive oxygen species through DPPH and ABTS assay. Around 70% activity was observed at 100 mg/ml concentration of the crude methanolic leaf extract of *M.randerianum* [30]. It has been investigated that the leaf extract showed potent radical scavenging capability with IC_{50} of 0.11 ± 0.5 mg/ml in DPPH assay and IC_{50} of 2.1±0.21 mg/ml in ABTS assay [31]. The IC_{50} values for quercetin, butylated hydroxyl toluene and ascorbic acid were 5.2 ± 1.24 μg/mL, 13.34 ± 1.21μg/mL and 30.32 ± 0.36μg/mL respectively in DPPH scavenging assay and the IC_{50} values of quercetin, ascorbic acid and butylated hydroxyl toluene were 6.13 ± 0.66 μg/ml, 76.4 ± 0.13 μg/ml and 19.2 ± 1.01 μg/ml respectively in ABTS scavenging assay. Rekha*et al*., [32] have established the antioxidant activity of tannins islaoted from the leaves of M. malabaricum through 1, 1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging

activity (EC50 of 2.67 µg/ml), Hydroxyl radical scavenging activity (EC50 of 7.73 µg/ml), Nitric oxide radical scavenging activity (EC50 of 11.3 µg/ml) and superoxide anion radical scavenging activity (EC50 of 19 µg/ml). This activity was expressed in EC50 value and compared with the standard, Butylated hydroxyl toluene (EC50 value 2.19µg/ml). Yashoda *et al* [33] has been evaluated radical scavenging potential of extract of two species of the genus Memecylon viz., *M. malabaricum* and *M. talboltianum*. They revealed that the leaf extract of *M. malabaricum* scavenged radicals more efficiently (IC_{50} 6.26µg/ml) than that of extract of *M. talboltianum* (IC_{50} 43.80µg/ml) when compared with the standard Ascorbic acid (IC_{50} 2.63µg/ml). But Sivu *et al*., [34] reported negligible free radical scavenging capacity (IC_{50} 207. 24 µg/ml) through DPPH assay when compared with the standard compound quercetin (10.16 ± 1.75 mg/ml).

Anti-inflammatory Activity

There is a report on a single novel compound namely, Memecylaene (4,9,14,19-Tetramethyl-1,6,11,16-tetraoxacycloeicosa-3,8,13,19-tetraene) from the leaves of this plant [35]. They carried out studies on the anti-inflammatory properties of Memecylaene (Figure 3) and also described about the anti-angiogenic, anti-proliferative, pro-apoptotic and anti-oxidant features in *In vivo* and *Ex vivo* experiments. The structure is given below in Figure 2.

Anti-diabetic Activity

The diabetes infected people have grown drastically in the modern world. Its treatment with some medicinal plants is very much appreciated. Ramaiah *et al*., [36] investigated the antidiabetic potential of the *M. malabaricum* leaves using experimental model of alloxan induced diabetes in rats and they revealed that the methanolic extract at the dose of 400 mg/kg body weight showed significantly decreased ($P<0.01$) of the raised blood glucose level, comparable to reference standard, gliclazide. The methanol extract of *M. malabaricum* showed the highest antidiabetic activity of 94.7% for α-amylase and 89% for α-glucosidase activity in the antidiabetic studies conducted among five different species of Memecylon by Bharathi and Prakash [37].

Anti-psoriatic Activity

The medicinal plants such as *Radix paeoniae* Alba.(Paeoniaceae), *Rubia cordifolia* Linn.(Rubiaceae), *Coptis chinensis* Franch.(Ranunculaceae), *Alpinia galangal* Linn.(Zingiberaceae), *Annona squamosa* Linn.(Annonaceae), *Curcuma longa* Linn. (Zingiberaceae), etc., are scientifically reported to be effective for the treatment of psoriasis [38]. Dhanabal *et al*., [39] evaluated in vivo antipsoriatic activity of aqueous extract, hydroalcoholic extract and their fractions of *M. malabaricum* leaves by mouse tail test and in vitro antipsoriatic activity using HaCaT cells, lipoxygenase inhibition and thymidine phosphorylase inhibition. They found out a significant ($P < 0.05$) reduction in epidermal thickness in the mouse tail test when compared with control except the hydroalcoholic extract. The chloroform fraction showed the maximum activity against HaCaT cells in their experiment.

Clastogenic Activity

The fragmentation of chromosomes or loss of chromatin in micronuclei is known as clastogenic effect. This clastogenicity is usually detected through micronucleus test. The genotoxic evaluation of the different concentration of petroleum ether, and ethanolic extract of four different plants (*Justicia simplex*, *Memecylon malabaricum*, *Litzea quinqueflora* and *Myxopyrum smilacifolium*) was carried out by Joseph *et al*., [40]. They conducted their studies on erythrocytes of *Zekaranakeralenisis tadpoles* using micronuclei test under controlled laboratory conditions after 24 and 48h of exposure. The leaves of *M. malabaricum* demonstrated comparable genotoxicity effect.

Anthelmintic Activity

The benefit of using medicinal plants for helmintic infection is recognised from the ancient period. The aqueous and ethanol extract of *Memecyclon malabaricum* leaves are reported to have potent anthelmintic activity when compared with the standard Piperazine citrate. Ramanjayalu *et al*., [41] revealed the significant anthelmintic activity at highest concentration of 60 mg/ml among three concentrations (20, 40, 60 mg/ml) of both aqueous and ethanol extract of *Memecyclon malabaricum* leaves. The Indian earthworm *Pheritimaposthuma* was used for this study.

Cytotoxic Evaluation

A number of cytotoxic agents from medicinal plants were screened and identified against cancer cell lines. RamyaSree and Thoppil [42] reported significant mitotic index and C-mitotic activity of the plant extract using *Allium cepa* assay in the 24 hour treatment. They revealed that the cytotoxic effect of this plant is due to the presence of potential amount of the phytoconstituents such as proteins, carbohydrates, alkaloids, flavonoids, terpenoids and phenolic compounds.

CONCLUSION

Memecylon randerianum is a notable medicinal woody shrub that can provide valuable active molecules for the treatment of several disorders. The present review gives a good opportunity for the drug discovery researchers. Based on the studies conducted so far, this plant could be effectively used for various kinds of human diseases such as diabetes, microbial infections, psoriasis, inflammations, skin diseases such as herpes and chickenpox. It can be say that it is definitely a pharmacologically promising plant for the future. So it is more suitable for pharmacological approach with the isolation of bioactive molecules of interest. This review also recommended to carry out further investigation on the molecular and clinical applications of this plant.

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REFERENCES

1. Suja A. Medicinal plants of Western Ghats. Sahyadri E. News: Issue XII http://www. ces. iisc. ernet. in/biodiversity/sahyadri_enews/newsletter/issue12/Index. htm. 2005.
2. Purushothaman, T., & Mol, K. I. (2020). Ethnobotanical Medicines Used by the Kani and Kurichiyar Tribal Communities of Kerala.
3. Serna, D. M. O., & Martínez, J. H. I. (2015). Phenolics and polyphenolics from Melastomataceae species. Molecules, 20(10), 17818-17847.
4. Nono, R. N., Barboni, L., Teponno, R. B., Quassinti, L., Bramucci, M., Vitali, L. A., ...&Tapondjou, A. L. (2014). Antimicrobial, antioxidant, anti-inflammatory activities and phytoconstituents of extracts from the roots of Dissotisthollonii Cogn.(Melastomataceae). *South African Journal of Botany*, *93*, 19-26.
5. Nzogong, R. T., Ndjateu, F. S. T., Ekom, S. E., Fosso, J. A. M., Awouafack, M. D., Tene, M., ...& Choudhary, M. I. (2018). Antimicrobial and antioxidant activities of triterpenoid and phenolic derivatives from two Cameroonian Melastomataceae plants: Dissotissenegambiensis and Amphiblemmamonticola. *BMC complementary and alternative medicine*, *18*(1), 159.
6. Simpson, M.G. 8—Diversity and Classification of Flowering Plants: Eudicots. In Plant Systematics, 2nd ed.;Simpson, M.G., Ed.; Academic Press: San Diego, CA, USA, 2010; pp. 275–448. ISBN 978-0-12-374380-0.
7. Amarasinghe, P., Joshi, S., Page, N., Wijedasa, L.S., Merello, M., Kathriarachchi, H., Stone, R.D., Judd, W., Kodandaramaiah, U. and Cellinese, N., 2021. Evolution and biogeography of Memecylon. *American Journal of Botany*, 108(4), pp.628-646.
8. Bremer, K., 1979. Taxonomy of Memecylon (Melastomataceae) in Ceylon. *Opera Bot.* 50: 1 – 32.
9. Bharathi, T. R., Sampathkumara, K. K., & Prakash, H. S. (2016). Memecylon species: A review of traditional information and taxonomic description. *Int J Phar Pharm Sci*, *8*, 1-9.
10. Ramasetty, B. T., Bajpe, S. N., Kadappa, S. K. K., Saini, R. K., Basavaraju, S. N., Ramachandra, K. K., & Sripathy, P. H. (2016). Identification and genetic diversity analysis of Memecylon species using ISSR, RAPD and Gene-Based DNA barcoding tools. *Electronic Journal of Biotechnology*, *24*, 1-8.

11. Mandan Jyothi, K. Bird community structure in the sacred groves of northern kerala (Doctoral dissertation, Department of Wildlife Sciences, College of Forestry, Vellanikkara).
12. Dhanabal, S. P., Muruganantham, N., Basavaraj, K. H., Wadhwani, A., & Shamasundar, N. M. (2012). Antipsoriatic activity of extracts and fractions obtained from *Memecylon malabaricum* leaves. *Journal of Pharmacy and Pharmacology*, *64*(10), 1501-1509.
13. Hullatti, K. K., & Rai, V. R. (2004). Antimicrobial activity of *Memecylon malabaricum* leaves. *Fitoterapia*, *75*(3-4), 409-411.
14. Ambasta, S.P.E.D., 2000. The useful plant of India, national institution of sci. *Communication, Delhi*, *239*.
15. Sanilkumar and MG, Geethu Krishna., 2019. Indigenous knowledge of coastline sacred groves in Central Kerala, India. *Indian Journal of Traditional Knowledge (IJTK)*, *18*(3), 541-546.
16. Iyengar, M. A., Nayak, S. G. K., & Singh, R. (1994). Folkloric uses of *memecyclon malabaricum* (CB cl.) cogn. *Ancient science of life*, *13*(3-4), 242.
17. Mohammed, F., & Shetty, P. S. (1887). Exploration and elucidation of various medicinal plants used in the management of dermatological disorders by folklore practitioners of Udupi District, Karnataka, India. Journal of Pharmacognosy and Phytochemistry, 8(1), 2019.
18. Asha, B., Krishnappa, M., & Kenchappa, R. (2015). Determination of Nutritive Value and Mineral Elements of Some Species of Genus Memecylon Linn. from Central Western Ghats. *Science, Technology and Arts Research Journal*, *4*(4), 58-64.
19. Ramaiah, M., Ganga, R. B., & Chakravarthi, G. (2013). Antidiabetic Activity of Methanolic Extract of *Memecylon malabaricum* Cogn (Melastomataceae) Leaves. *International Journal of Pharma and Bio Sciences*, *4*, 822-828.
20. Rekha, N. D., Gowda, T. V., Aradhya, S. M., Suresha, R. N., & Jayashree, K. (2014). Anti-inflammatory properties of Memecylaene: A novel compound isolated from *Memecylaene malabaricum*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *5*, 1645-1654.
21. Rajalakshmi, C. (2018). GC MS analysis of the leaves of *Memecylon malabaricum*. Journal of Pharmacognosy and Phytochemistry, 7(5), 2155-2157.
22. Silva, N. C. C., & Fernandes Júnior, A. (2010). Biological properties of medicinal plants: a review of their antimicrobial activity. *Journal of venomous Animals and Toxins including tropical diseases*, *16*(3), 402-413.
23. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582.
24. Hegde, N. P., & Hungund, B. S. (2020). Isolation, identification and in vitro biological evaluation of phytochemicals from *Memecylon randerianum*: a medicinal plant endemic to Western Ghats of India. *Natural Product Research*, 1-5.
25. Sekhar, S., Sampath Kumara, K. K., Niranjana, S. R., & Prakash, H. S. (2015). In vitro antioxidant activity, lipoxygenase, cyclooxygenase-2 inhibition and DNA protection properties of Memecylon species. International Research Journal of Pharmacy and Pharmaceutical Sciences. 5(2), 257-262.
26. Bharathi, T. R., Madhusudhan, M. C., Kumar, P. P., Nayaka, C. S., & Prakash, H. S. (2015). Antimicrobial potential of *Memecylon* L. species from Western Ghats against clinical isolates of pathogenic bacteria. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, *6*(4), 1280-1287.
27. Sivu, A.R., Pradeep, N.S., Rameshkumar, K.B. and Pandurangan, A.G., 2013. Evaluation of phytochemical, antioxidant and antimicrobial activities of Memecylon L. species from Western Ghats. *Indian Journal of Natural Products and Resources*, 4(4), 363-370
28. Hullatti, K.K. and Rai, V.R., 2004. Antimicrobial activity of *Memecylon malabaricum* leaves. *Fitoterapia*, 75(3-4), pp.409-411.
29. John, J., Ragi, P.R., Sujana, K.A. and Kumar, N.A., 2014. Antifungal activity of selected plant extracts on *Peridiopsoramori* causing brown rust in mulberry, *International Journal of Pharmacognosy*.1(9), 587-591
30. Hegde, N. P., & Hungund, B. S. (2020). Isolation, identification and in vitro biological evaluation of phytochemicals from *Memecylon randerianum*: a medicinal plant endemic to Western Ghats of India. *Natural Product Research*, 1-5.

31. Sekhar, S., Sampath Kumara, K. K., Niranjana, S. R., & Prakash, H. S. (2015). In vitro antioxidant activity, lipoxygenase, cyclooxygenase-2 inhibition and DNA protection properties of Memecylon species. International Research Journal of Pharmacy and Pharmaceutical Sciences. 5(2), 257-262.
32. Rekha, N. D., Aradhya, S. M., & Jayashree, K. (2015). The antiangiogenic, antioxidant and proapoptoticchemo preventive properties of tannins from *Memecylon malabaricum* (Cl.). *International Journal of Pharmaceutical Sciences and Research (IJPSR)*, *6*(1), 259-266.
33. Yashoda, K., Prashith, T. R., Manasa, M., & Raghavendra, H. L. (2014). Antimicrobial and Radical Scavenging Activity of *Memecylon malabaricum* Memecylon *talboltianum* Brand is. *Science, Technology and Arts Research Journal*, *3*(2), 174-179.
34. Sivu, A.R., Pradeep, N.S., Rameshkumar, K.B. and Pandurangan, A.G., 2013. Evaluation of phytochemical, antioxidant and antimicrobial activities of *Memecylon* L. species from Western Ghats. *Indian Journal of Natural Products and Resources*, 4(4), 363-370
35. Rekha, N. D., Gowda, T. V., Aradhya, S. M., Suresha, R. N., & Jayashree, K. (2014). Anti-inflammatory properties of Memecylaene: A novel compound isolated from *Memecylaene malabaricum*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *5*, 1645-1654.
36. Ramaiah, M., Ganga, R. B., & Chakravarthi, G. (2013). Antidiabetic Activity of Methanolic Extract of *Memecylon malabaricum* Cogn (Melastomataceae) Leaves. *International Journal Of Pharma And Bio Sciences*, *4*, 822-828.
37. Bharathi, T. R., & Prakash, H. S. (2017). Comparative evaluation of antidiabetic and antioxidant potency of different extracts obtained from *Memecylon* species. *Int J Pharm PharmSci*, *20179*, 187-91.
38. Singh, S. K., Chouhan, H. S., Sahu, A. N., & Narayan, G. (2015). Assessment of in vitro antipsoriatic activity of selected Indian medicinal plants. *Pharmaceutical biology*, *53*(9), 1295-1301.
39. Dhanabal, S. P., Muruganantham, N., Basavaraj, K. H., Wadhwani, A., & Shamasundar, N. M. (2012). Antipsoriatic activity of extracts and fractions obtained from *Memecylon malabaricum* leaves. *Journal of Pharmacy and Pharmacology*, *64*(10), 1501-1509.
40. Joseph, L., 2016. Cytotoxic potential of selected South Indian Medicinal Plants., *World Journal of Pharmaceutical Sciences*,4(3), 295-298.
41. Ramanjayalu, A., Uwais, M., Nithyananda, G. S., Satish, S., Shalavadi, M., & Biradar, B. S. (2012). A study on in vitro anthelmintic activity of aqueous and alcoholic extracts of *Memecyclon malabaricum* leaves against Pheritimaposthuma. *Int J Pharm ChemSci*, *1*, 621-4.
42. Ramya Sree, P. R. and Thoppil, J. E. (2018). Cmitotic potential of aqueous leaf extract of *Memecylon randerianum* SM & MR Almeida: A promising natural colchicine analog. International Research Journal of Pharmacology 9(11):115-118 *3*(2), 174-179.

Table 1.Physico-chemicalProximates and Nutritive value of the leaves [18]

Sl No	Physico-chemical parametres	Average value
1.	Ash (in %)	4.60±0.92
2.	Moisture content (in %)	32.00±1.90
3.	Crude fat (in %)	4.78±0.25
4.	Crude fibre (in %)	24.2±0.86
5.	Crude Protein (in %)	2.23±0.14
6.	Carbohydrate (in %)	27.91±1.77

Table 2. Elemental composition in the leaves [18]

Sl No	Macroelements	Average value (in ppm)
1.	Sodium	65
2.	Potassium	5642.90
3.	Phosphorus	512.40

4.	Calcium	6677.80
5.	Magnesium	1861.28
	Microelements	**Average value (in ppm)**
1.	Zinc	12.60
2.	Copper	1.730
3.	Manganese	188.59
4.	Iron	188.59
	Heavy metals	**Average value (in ppm)**
1.	Lead	<0.01
2.	Cadmium	<0.01

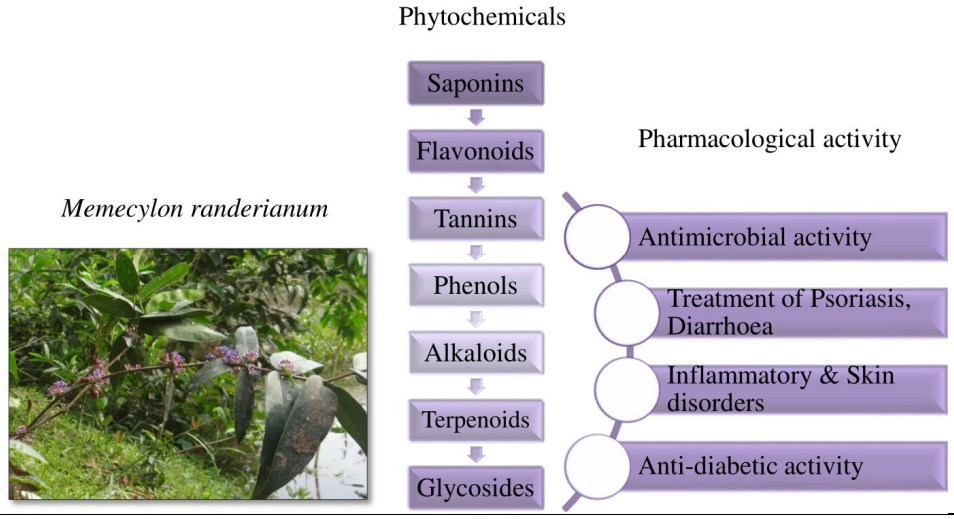

Figure 1: showing the bioactive phytochemicals and medicinal properties of *Memecylon randerianum*

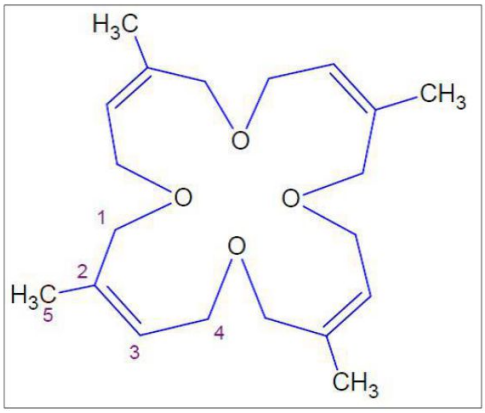

Figure 2: Structure of Memecylaene[35]

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RESEARCH ARTICLE

Adsorption of Heavy Metal Cu^{2+} from Synthetic Waste Water using Chitosan encapsulated Iron Oxide Nanoparticles

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ABSTRACT

Industrial waste water contains large number of toxic metals. There are at least 20 metals which cannot be degraded or destroyed which includes Cu, Ni and Zn. Several methods were employed to remove and recover such metals from our environment to remove the toxic metal from waste water. Adsorption is one of the alternatives for such cases and is an effective technique used in industry especially in water and waste water treatments. Cost is an important parameter for comparing the adsorbent materials. Therefore, there is increasing research interest in using alternative low –cost adsorbents. In the present study Chitos an encapsulated Iron Oxide Nanoparticles are used as an adsorbent for removing heavy metal ions from aqueous solutions. The experiment results showed that maximum removal of copper ion by chitos an encapsulated iron oxide nanoparticles occurs at optimum condition such as adsorbent dosages 3.5gm, P^H 6, initial metal concentration 10mg/l.

Key words: $CuSO_4$, $5H_2O$, SEM, EDAX, Chitosan, Adsorption

INTRODUCTION

Heavy metals are produced in large amounts during industrial activities and contaminate the environment. Metal ions are Non-biodegradable and many of them are soluble in aqueous media and easily available for living organisms. Heavy metals account for a number of disorders in plants and animals and their removal from aqueous

media is an important and challenging task [1,2]. Revelation to heavy metals, even at trace levels, is harmful to human beings [3–6]. Thus, removal of undesirable metals from water sources is considered as an important task that is still threatening the environment. As the tolerable limit of lead in drinking water is 0.05 mg[7], the presence of excess lead ions in drinking water causes severe diseases such as anaemia, encephalopathy, and hepatitis. Lead ions are characterized by an eager affinity towards ligands containing thiol and phosphate groups that inhibit, in turn, the biosynthesis of heme, causing physiological damage to both the kidney and liver; similar to that of calcium. Numerous methods are reported as efficient for the removal of heavy metal ions from water sources, including chemical precipitation, ion exchange, adsorption, membrane filtration and electrochemical technologies [8–13]. Therefore, the adsorption process is considered as one of the major suitable technique for heavy metals removal from water/wastewater sources. Currently, nanosized metal oxides (NMOs), including nanosized ferric oxides, manganese oxides, aluminium oxides, titanium oxides, magnesium oxides and cerium oxides, are classified as promising adsorbents for heavy metal removal from aqueous systems [14–16]. In the present work, systematic laboratory investigations for the removal of Cu (11) ions from aquesous solution through adsorption by chitosan – iron oxide nanoparticles.

MATERIALS AND METHODS

Synthesis of Chitosan Nano Particles

Chitosan is synthesised by using deacetylation method. Then about 200 mg 85% deactylated chitosan powder and Iron oxide were made in to a gel using 2-5ml of formaldehyde. The mixture was stirred magnetic stirrer using for 1 hour finally chitosan encapsulated Iron oxide nanoparticle obtained.

SEM / EDX Analysis

The sample was sprinkled on a double side carbon tape and mounted on aluminum stubs that were placed inside an airtight chamber. The selected portion was analyzed through SEM(Carl Zeiss EVO 18 special edition microscopy) and EDX under ultra high vacuum (Oxford instruments energy dispersive X-ray micro analysis system) to examine morphological and elemental composition.

Adsorption studies

The adsorption of heavy metals on chitosan-iron oxide nanoparticles was studied by batch technique. A known weight 0.5g, 1g, 1.5g, 2, 2.5g and 3g of chitosan – iron oxide adsorbent was equilibrated with 20 ml of the heavy metal Cu solution of known concentration 10, 20, 30, 40, 50, and 60 ppm respectively in six stoppered borosil glass flask at a fixed temperature at 30^{o}c in an orbital shaker for a known period of 360 min. After equilibration, collect sample from each flask and suspension of the adsorbent was separated from solution by filtered using whatman filter paper No 1. The concentration of heavy metal ions in solution was measured by UV Visible spectrophotometer. The effect of several parameters such as P^{H}, concentration and adsorbent dose on the adsorption was studied.

The percentage heavy metal removal was calculated using EQ metal ion concentration (%) $=\frac{Co-Ce}{Co}$ x 100

Where C_0-initial metal ion concentration of test solution mg/l, Ce-final equilibrium concentration of test solution[17].

RESULTS AND DISCUSSION

SEM / EDAX Studies

The scanning Election Microscope (SEM) images shown in Fig 1(a), and 1(b) shows the nature of the modified adsorbent before and after adsorption. Fig 1(a) shows unoccupied pores on the adsorbent before adsorption while Fig 1(b) shows the morphology of the adsorbent after the loading of Cu (II) ions. Fig 1(b) shows the outer pores are covered by Cu (II) ions.

EDAX

Energy dispersive X-ray Spectroscope is used to analysis data confirms the elements of the material.

EFFECT OF P^H

The adsorption studies of copper on chitosan encapsulated iron oxide nanoparticles is carried out at P^H varying between 2 to 8 and the adsorbent dosageof 1g, concentration at 20ppm and contact time of 360 minutes. The percentage of elimination of copper by chitosan nanoparticles increases with the increase in P^H and reach a maximum value of 93% at P^{H6}. The percentage of copper removal were minimum with a percentage of 75.67% when the P^H is 2. Fig3. indicates that the P^H has a significant effect and influences the adsorption of copper. This shows that at low P^H the ions hardly adsorb to the surface because it is already saturated by H^+ ions by increasing the P^H, there will be an increase in the adsorption capacity since the reaction between the H^+ ions in the solution and copper (II) metals decreases and consequently the cu (II) metal ions can readily adsorb to the surface of chitosan encapsulated iron oxide nanoparticles, More over for a high P^H, the Cu (II) ion precipitate, and consequently the adsorption capacity decreases [18].

Effect of adsorbent dosage

The results for adsorptive of heavy metals with respect to adsorbent dose are show in Fig 4. The percentage removal of heavy metals were seen to increase with adsorbent dose. It is observed that there is a sharp increase in percentage removal with adsorbent dose for Cu (II) ions. The maximum removal of Cu is 90% at 3.5gm dose amount of chitosan encapsulated ion oxide nanoparticles adsorbent remains parameters are constant. It is shown that the percent removal of heavy metals increases rapidly with increase in the dose of the adsorbents due to the greater availability of the exchangeable sites or surface area

Effect of initial metal ion concentration

The effect of varying initial cu (II) ion concentrations (10-70mg/l) on the adsorption capacity of the adsorbent was studied at constant adsorbent dose P^H and contact time. The results are shown in Fig 5.which indicates that the percentage removal decreases with the increase in initial metal ion concentration. This is because there were no more adsorption sites on the adsorption surface of the adsorbent material the maximum removal of Cu (II) using chitosan encapsulated iron oxide nanoparticles at 92% at copper ion concentration 10mg/l [19].

CONCLUSIONS

Chitosan is a cheap and effective adsorbent for the removal of Cu from waste water. I this present study its reveals the maximum removal of copper ion by chitosan iron oxide nanoparticles occurs at optimum condition such as P^H 6, adsorbent dosage 3.5g and concentration 10mg/l. From this it is concluded that the Chitosan Nano particles can be used as removal agent if excessive copper content from the water.

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REFERENCES

1. S Jiwan, AS Kalamdhad. Effects of heavy metal on soil, plants, human health and aquatic life, a reciew[J].Int.J.Res.chem. Environ,2011,1(2):15-21.

2. M Sprynskyya, BuszewskiAP, Terzzyk, J Namiesnik. Study of the selection mechanism of heavy metal (Pb^{2+}, Cu^{2+},Ni^{2+} and cd^{2+}) adsorption on dinontilolote[J]. J.Coll.Interf.Sci,2006,304, 21-28.
3. M Jamil, MS Zia, MQasim. Contamination of Agro-Ecosystem and Human Health Hazards from Wastewater Used for Irrigation[J]. J. Chem. Soc. Pak,2010, 32, 370–378.
4. S Khan, Q Cao, YM Zheng, YZ Huang, YG Zhu.Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China[J]. Environ. Pollut,2008, 152, 686–692.
5. A Singh, RK Sharma, M Agrawal, FM Marshall. Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India.[J]. Food Chem. Toxicol,2010, 48, 611–619.
6. SH Peng, WX Wang, XD Li, YF Yen. Speciation of heavy metals in surface sediments from Suzhou Creek[J]. Chemosphere,2004, 57, 839–851.
7. Guidelines for Drinking-Water Quality, 2nd ed., Vol. 1, World Health Organization, Geneva, 1998.
8. FL Fu, Q Wang.Removal of heavy metal ions from wastewaters: A review[J]. J. Environ. Manage. 92 (2011) 407–418.
9. YH Wang, SH Lin, RS Juang, J Hazard. Removal of heavy metal ions from aqueous solutions using various low-cost adsorbents[J]. Mater.,2003,102, 291–302.
10. DW O'Connell, C Birkinshaw, TF O'Dwyer. Heavy metal adsorbents prepared from the modification of cellulose: a review. [J].Bioresour. Technol.,2008,99, 6709–6724.
11. TA Kurniawan, GYS Chan, WH Lo, S Babel.Physico–chemical treatment techniques for wastewater laden with heavy metals[J]. J. Chem. Eng.,2006, 118,83–98.
12. N Galil, M Rebhun. Chemically Enhanced Wastewater Treatment: An Appropriate Technology for the 1990's[J]. Water Sci. Technol.,1990, 22, 203–210.
13. M Eid, AMA Omar, AMDessouki[C]//. Recovery of Chromium Ions From Electroplating Wastewater by Flotation Gamma Irradiation and Adsorption onto Hydrogels, 8th Arab International Conference On Polymer Science & Technology, EGYPT,2005,27–30.
14. JE Vanbenschoten, BE Reed, MR Matsumoto, PJ McGarvey. Metal Removal by Soil Washing for an Iron Oxide Coated Sandy Soil.[J]. J. Water Environ. Res.,1994, 66, 168–174.
15. JA Coston, CC Fuller, JA Davis.Pb2+ and Zn2+ adsorption by a natural aluminum- and iron-bearing surface coating on an aquifer sand[J].Geochim. Cosmochim. Acta, 1995, 59, 3535–3547.
16. A Agrawal, KK Sahu.Kinetic And isotherm studies of cadmium adsorption on manganese nodule residue[J]. J. Hazard. Mater.,2006, 137, 915–924.
17. M El, S Abd el Raouf, AM Abd El Raheim, AF El Kafrawy, N El, S Maysour, A KhIbraheim, AA Abdel.Adsorption of heavy metal ion from aqueous solution by nickel oxide nano catalyst prepared by different methods[J]. Int. J. Chem. Mater. Sci., 2013, 2, 036–044.
18. Babel S, Kurniawan TA. Low Cost adsorbents for heavy metals uptake from contaminated water:a review[J].J.Hazard mater, 2003, 97, 219-243.
19. Lokendra Singh Thakur, Mukesh Parmar. Adsorption of Heavy metal (Cu^{2+}, Ni^{2+} and Zn^{2+}) from synthetic waste water by Tea waste Adsorbent[J]. International Journal of Chemical and Physical Sci, 2013, 2(6), 6-19.

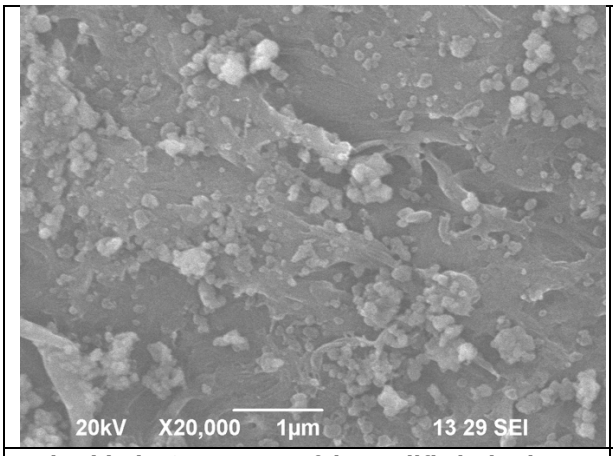

Fig 1(a) The SEM Image of the modified adsorbent before adsorption

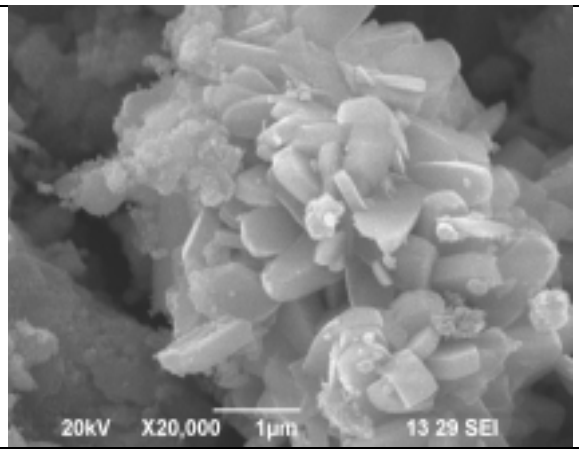

Fig 1(b) The SEM Image of the modified adsorbent after adsorption

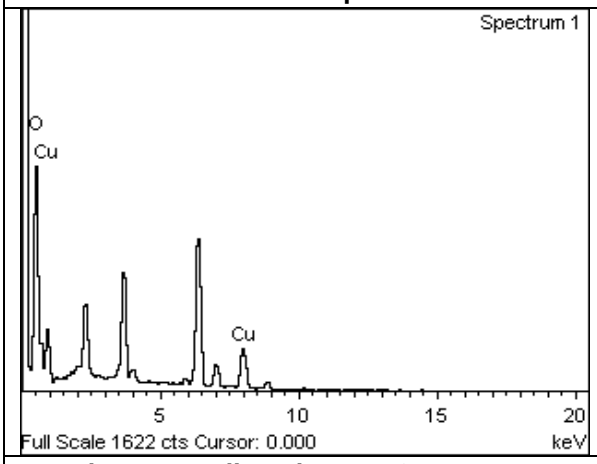

Fig 2. Energy dispersive X-ray Spectroscopy

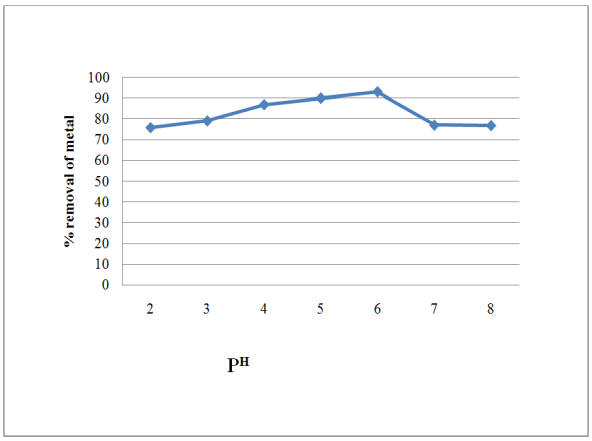

Fig 3 Effect of P^H on adsorption of Cu

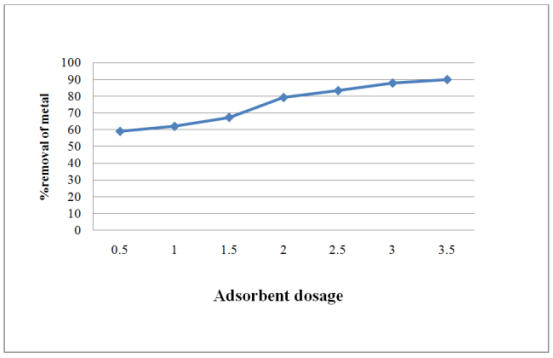

Fig.4. % removal of metal against adsorbent dosage

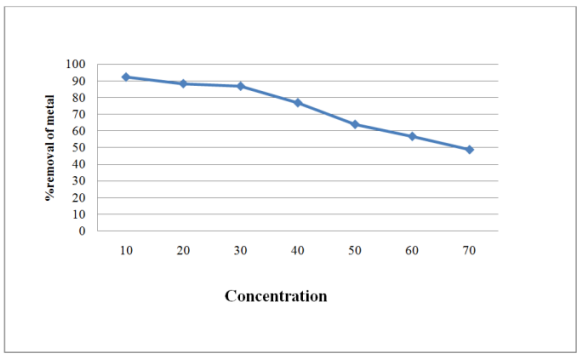

Fig 5. %removal of metal against concentration

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RESEARCH ARTICLE

Pharmacognostic Study of a Polyherbal Formulation Containing Four Native Indian Medicinal Plants

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ABSTRACT

In today's world standardization of drugs and formulation is very important parameter for drug discovery. Our study includes pharmacognostic study of a polyherbal formulation comprising of four native Indian plants i.e. *Pterocarpus marsupium*, *Salacia reticulata*, *Tinospora cordifolia* and *Vetiveria zizanioides*. Morphological parameters are a great insight in identifying and understanding the plant. In our study all the morphological parameters were covered. Microscopical studies helped us to unravel the basic anatomy of the plants and helped to identify the plant accurately. Powder microscopy is an important tool which is performed here to correctly understand the drug and to find out if any adulteration is done. Physico-chemical parameters like ash value and extractive value helped us to match with the pharmacopeial standards. Along with all these parameters, phytochemical screening helped us to identify all the phytochemicals the plants in this formulation possesses. These methods become a powerful tool to uncover and check even a very small amount of adulteration in a large extent. All the result of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the drugs. These methods will be useful for standardization of different formulations also.

Keywords: Morphology, Microscopy, Ash Value, Phytochemical screening.

INTRODUCTION

Home grown medications have been utilized since ancient times as pharmaceuticals for the treatment of a scope of ailments. Plants have assumed a key part on the human wellbeing. Regardless of awesome advances and cutting edge technology development in late decades, plants still make vital commitment to medicinal services. It is assessed that 25% of every single prescription written in present day are from higher plants [1]. According to World Health Organization (WHO), because of poverty and absence of access to modern drug, around 65-80% of the total population which lives in developing nations depends particularly on plants for essential medical treatment [2].

Need of Standardization

Every Herbal Formulation must be standardized as per WHO guidelines. The target of WHO rules is to characterize essential criteria for the assessment of value, security and viability of medications and home grown solutions. India is one of the world's twelve driving biodiversity focuses with the nearness of more than 45,000 distinctive plant species, out of this around 15,000-20,000 plants have great restorative properties of which just around 7,000-7,500 are being utilized by conventional specialists. The Siddha arrangement of solution uses around 600, Ayurveda 700, Unani 700 and cutting edge prescription around 30 plants species. Projection is being made that after data innovation, natural innovation will be India's greatest income worker. India has an extraordinary part to play, as provider of home grown items to meet the residential needs, as well as to exploit the gigantic fare potential. To be a worldwide provider of home grown solutions by fitting in with global determination the accompanying angles requests significant consideration:-

- Proper plant ID of every single therapeutic plant in Indian System of Medicine. Every single home grown fixing in readiness to be determined by their herbal names other than their mainstream/regular names.
- Processing of medicinal plants in a logical, financial and safe way utilizing comparable ones utilized for advanced medications.
- Isolation and substance portrayal of intense fixings including inorganic constituents, wherever conceivable.
- Pharmacological and clinical studies to determine their viability and security.
- Standardization to guarantee consistency. The utilization of therapeutic plants in blend to be restricted to encourage examination and to apply quality control and institutionalization parameters to home grown medication arrangements.
- Documentation of research [3].

Standardization of herbal drugs and formulation incorporates operations for evaluation of purity, quality and quantity of active constituents. According to European Medicines Agency (EMEA), the herbal markers are known as constituents of herb with which concentration of phytoconstituents can be assessed. The amount of a marker in a specific herbal preparation can be utilized as a pointer of its quality [4].

Need of Study

At present, serious resistance and side effects has been developed for various allopathic medicines for various diseases. This highlights the importance of searching for an alternative disease therapy strategy with drugs which have minimal or no side effects. Development of a suitable formulation along with its complete evaluation shall make the study useful for medical as well as commercial utility

MATERIALS AND METHODS

Collection

The selected herbs have been collected from following Places:-

- Payneer Ayurvedical, Irinjalakuda, Thrissur, Kerala.

- Kodivalapalapi Gardens, Vellenjor, Thrissur, Kerala.
- Thumboormuzhy Garden, District Tourism Promotion Council, Thumboor, Thrissur, Kerala.
- Pullukara House, Kottenloor, Irinjalakuda, Thrissur, Kerala.

Morphology

Plainly visible personality of a plant material depends on shape, measure, shading, taste, surface qualities, surface, crack trademark and appearance of cut surface. The size, shape and relative places of the diverse cells and tissues are likewise decided infinitesimally [5,6].

Microscopy

Minute examination of segment supported by stains helps in qualification of life structures in adulterants. Transverse segments of all the plant parts which are utilized as a part of the definition were cut with the assistance of surgical blades. Segments were mounted with glycerine in the slide and care has been taken to maintain a strategic distance from entanglement of air rises in the slide. Saffranin is utilized as a recoloring specialist to recognize distinctive tissues in the transverse segment of the plants. The size, shape and relative places of the diverse cells and tissues are resolved. The essential course of action of tissues in every medication is decently constants like filaments, sclereids, tracheids, vessels and stopper are slightest influenced by drying [5,6].

Powder Microscopy

Microscopic examination of segment and powder drugs supported by stains helps in qualification of life systems in adulterants. Further, microscopical examination of epidermal trichomes and calcium oxalate precious stones is to a great degree profitable, particularly in powdered medications, as the cells are in all likelihood broken aside from lignified cells. The cell substance, for example, starch granules, calcium oxalate precious stones, aleurone grains and so on are scattered in the powder. A few pieces are particular for every powder, which may comprise of parts of cells or gathering of cells. The size shape and relative places of the distinctive cells and tissues, substance nature of the cell dividers and of the cell substance are resolved. The fundamental course of action of tissues in every medication is genuinely steady like filaments, sclereids, tracheids, vessels and stopper are slightest influenced by drying. Starch granules, calcium oxalate precious stones, epidermal trichomes and lignin are inspected deliberately [5,6].

Phytochemical Screening

The drugs were collected and shade dried at room temperature for two to three weeks, pulverized and sieved the powder to separate coarse powder and fine powder. 500 g per batch of shade-dried coarse powder of the drugs was successively extracted with petroleum ether (60-80), chloroform and ethanol (95%). The ethanolic extract of all the drugs and the formulation were evaporated to dryness under reduced pressure and controlled temperature (40-50ºC). The extracts were treated with distill water and filtered. The filtrates were subjected to qualitative chemical tests to detect the presence of various phytoconstituents [7].

Tests for Alkaloids

Dragendroff's test

To the filterate Dragendroff's Reagent (potassium bismuth iodide) was added. A reddish brown precipitate indicated the presence of alkaloid.

Mayer's test

To the filterate Mayer's Reagent (Potassium mercuric iodide) was added. A cream precipitate indicated the presence of alkaloid.

Wagner's test

To the filterate Wagner's Reagent (Iodine in potassium iodide) was added. A reddish brown precipitate indicated the presence of alkaloid.

Hager's test

To the filterate Hager's Reagent (saturated picric acid solution) was added. A yellow precipitate indicated the presence of alkaloid.

Tests for Sugars

Molisch test

2-3 drops of alfa naphthol were added in test solution. Then few drops of conc. H_2SO_4 were added from the side of test tube. Violet colour ring was formed at the junction of two layers, indicated the presence of carbohydrates.

Fehling's test

Fehling A solution and Fehling B solution were mixed in equal amount and boiled for one minute. Equal amount of test solution was added. It was again heated for 5-10 minutes. First yellow and then a brick red colour appearance indicated the presence of reducing sugars.

Benedict's test

Equal volume of test solution and Benedict's reagent was mixed. It was heated in boiling water bath for 5-10 minutes. Yellow, red or green colour was appeared, depending on the content of sugar.

Tests for Glycosides

Cardiac Glycosides

Keller-killiani Test (for deoxy sugar)

1 ml of glacial acetic acid containing traces of ferric chloride and 1 ml of concentrate sulphuric acid were added to the extract carefully. A reddish-brown colour formed at the junction of two layers and the upper layer turned bluish green indicated the presence of glycosides.

Legal test (for cardinolides)

Concentrated ethanolic extract was made alkaline with few drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside solution was added to the solution. Presence of blue coloration indicated the presence of glycosides in the extract.

Baljet test

Picric acid is added to the extract and made alkaline, gives a stable orange color in presence of glycosides.

Anthraquinone Glycosides

Borntrager's test

To the powdered drug or dried extract 1ml of dilute hydrochloric acid is added and heated for five minutes, and filtered while hot, to this add an equal volume of chloroform, shake well and collect the lower organic layer of chloroform, add ammonia half of its volume, shake well, lower ammonical layer produce rose pink colour after few minutes.

Modified Borntrager's test

Add 5 ml of 5% ferric chloride and 5 ml of dilute hydrochloric acid to the 5 ml of extract and boil for 5 min cool and add benzene or chloroform ,shake well, separate the organic layer and add equal volume of dilute ammonia, ammonical layer shows pink colour.

Tests for Saponins

Foam Test

A few mg of the test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. It is a stable, characteristic honeycomb like froth is obtained, saponins are present.

Tests for Tannins and Phenolic Compounds

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagents.

Ferric chloride reagent

Ferric chloride solution was added to a little of the above filtrate. If dark green or deep blue colour is obtained, tannins are present.

Lead acetate test

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. If precipitate is obtained, tannins are present.

Potassium dichromate test

If on addition of a solution of potassium dichromate in a test filtrate, dark colour is developed, tannins are present.

Tests for Flavonoids

Ammonia Test

Filter paper strips were dipped in the alcoholic solution of the extract and ammoniated. The filter paper strips turned yellow due to the presence of flavonoids. [5,6].

Physicochemical Studies

Ash Value

Ash Value was performed for the assurance of inorganic materials, for example, carbonates, silicates, oxalates and phosphates. Warming causes the loss of natural material as CO_2 deserting the inorganic segments. Fiery debris esteem is a vital normal for a medication and with the assistance of this parameter we can identify the degree of debasement and in addition build up the quality and immaculateness of the medication. The corrosive insoluble powder comprises principally of silica and high corrosive insoluble fiery debris consequently showing the sullying with natural material [5,6].

Whole ash

Powdered medication (2 gm) was burned in a tarred silica pot, spreading the material in an even layer, and lights it by progressively expanding the warmth to 500-600 ^oC until it is white, showing nonappearance of carbon. It is then cooled in a desiccator for 30 minutes, and weighed. Substance of aggregate fiery debris in milligram per gm of air dried material is computed [5,6, 8].

Acid insoluble ash

Total ash is bubbled tenderly with 25 ml weaken hydrochloric acid (6N) for five minutes. The insoluble matter gathered on fiery remains less channel paper washed with heated water until the filtrate is unbiased and lighted at a temperature of 500-600 ^oC to a steady weight. Substance of corrosive insoluble slag in milligram per gm of air dried material is ascertained [5, 6, 8].

Extractive values

The measure of a concentrate that a medication yields in a specific dissolvable is frequently a surmised measure of the measure of specific constituents that the medication contains. The petroleum ether remove contains settled oil, gums and unstable substances and then extracted successively by alcohol and water [5, 6, 8].

RESULTS AND DISCUSSION

Morphological study

Detailed morphological studies were done and photographed, giving the general appearance of the crude drugs. The morphological characters of the four plants are summarized from Table 1 to Table 4.

Microscopical Characters

Detailed microscopic studies were done and photographed, giving the general appearance of the internal structure of the crude drugs. Transverse section of *Salacia reticulata* root shows the presence outer cork, inner Cork, oil glands, medullary rays, xylem vessels as shown in Figure 1. Transverse section of *Tinospora cordifolia* stem shows the presence of cork, collenchymas, parenchyma, medullary rays, sclerenchyma, phloem, xylem parenchyma, xylem vessels, medullary rays and pith as shown in Figure 2.Transverse section of *Pterocarpus marsupium* shows the presence of pith, medullary rays and vessels as shown in Figure 3. Transverse section of *Vetiveria zizanioides* root shows the presence of epidermis, hypodermis, aerenchyma strands, schlerenchymatous cortex and aerenchymatous pith as shown in Figure 4.

Powder Microscopy

Microscopic examination of segment and powder drugs supported by stains helps in identifying characters like parenchyma, collenchymas, sclerenchyma, vascular bundles, starch grains and other powder characteristics.Microscopic section of Salacia reticulata shows the presence of fibres with starch in Figure 5. Microscopic section of Pterocarpus marsupium shows the presence phloem fibres in Figure 6. Microscopic section of *Tinospora cordifolia* shows the presence uniseriate medullary rays in Figure 7. Microscopic section of *Pterocarpus marsupium* shows the presence bordered pitted vessel in Figure 8.

Phytochemical Screening

The filtrates were subjected to qualitative chemical tests to detect the presence of various phytoconstituents. Alcoholic extracts of all the drugs when tested with various reagents shows the presence of alkaloids, glycosides, tannins flavonoids, etc. as shown in Table 5.

Physicochemical Parameters

All the ingredients shown total ash, acid insoluble ash and water soluble ash values within the specification limits mentioned in Ayurvedic Pharmacopoeia. The drug was extracted (Cold, Hot and Successive) with range of solvent (petroleum ether, chloroform, methanol and water) and maximum soluble extractive value was observed in water and methanol indicating the presence of more polar constituents (glycosides, carbohydrates, flavonoids, proteins, alkaloids etc.). All the values are within the specification limits mentioned in Ayurvedic Pharmacopoeiaas shown from Table 6 to Table 9.

CONCLUSION

Establishing standards is an integral part of establishing the correct identity, purity and quality of crude drugs and formulations. Majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physico-chemical parameters. Our study has shown that all the ingredients have passed the test of identity, purity and quality according to the Pharmacopoeial standards with the help of

morphological study, microscopical study, powder microscopy and physicochemical parameters. In addition our present result will pave the way for conducting more instrumental standardization parameters on the formulation. Further investigation into the bioactivity of the individual drug and the formulation may prove useful in the prevention of a variety of pathological condition. Isolation of the bioactive components through different chromatographical methods can lead to preclinical and clinical testing of the formulation.

REFERENCES

1. Fransworth NR, Morris RW. Higher plants-The sleeping giant of drug development. American Journal of Pharmaceutical Education 1976; 148: 46-52.
2. Akerale O, Heywood V, Synge H. The Conservation of Medicinal Plants. Cambridge University Press, Cambridge, UK 1991.
3. Jain JB, Sheetal CK, Bhattacharya S. Medicinal flora of Madhya Pradesh and Chhattisgarh-A Review. Indian Journal of Tradition Knowledge 2006; 5: 237-242.
4. Kushwaha SKS, Kushwaha N, Maurya N, Rai AK. Role of markers in the standardization of
5. herbal drugs: A review. Archives of Applied Science Research 2010;2 (1): 225- 229.
6. Anonymous. Indian Pharmacopoeia. Govt. Of India, Ministry of Health and Family Welfare, New Delhi Part-II 1996:53-55.
7. W.H.O. Quality control methods of medicinal plant material. Geneva 1998:10-34
8. Kokate CK, Purohit AP, Gokhale SB. A Text Book of Pharmacognosy, Nirali prakashan, Pune 2005
9. Mukherjee PK. Quality Control of Herbal Drugs "An approach to Evaluation of Botanicals". Horizons Business Pharmaceutical Publishers, New Delhi 2005:186-191.

Table 1: Morphological Characters of *Salacia reticula*

S.No.	Parameters	Root
1.	Odour	Characteristic
2.	Taste	Bitter
3.	Size	10-15cm
4.	Shape	Irregular shaped
5.	Touch	Hard
6.	Colour	Bark-Yellow Heartwood-Light Brown

Table 2: Morphological Characters of *Pterocarpus marsupium*

S.No.	Parameters	Heartwood
1.	Odour	Odourless
2.	Taste	Characteristic
3.	Size	12-20cm
4.	Shape	Log pieces
5.	Touch	Hard
6.	Colour	Light Brown

Table 3: Morphological Characters of *Tinospora cordifolia*

S.No.	Parameters	Stem
1.	Odour	Odourless
2.	Taste	Bitter
3.	Size	Long and lengthy uptu 5 meterwith 2-4cm in diameter
4.	Shape	Cylindrical, slender, slightly twisted
5.	Touch	Outer bark is thin and papery with rough surface
6.	Colour	Brown to greyish

Table 4: Morphological Characters of *Vetiver zizanioides*

S.No.	Parameters	Root
1.	Odour	Aromatic and very characteristic, smells like wet soil
2.	Taste	No specific taste
3.	Size	Fibre tufts upto 30 cm in length and 4-6mm in diameter
4.	Shape	Cylindrical fibres
5.	Touch	Smooth with short fracture
6.	Colour	Light brown

Table 5: Phyto-chemical Screening of ethanolic extract of drugs

Phytoconstituents	Phytoconstituents and Reagents		SR	PM	TC	VZ
Alkaloids			+	+	+	-
Carbohydrates	Molisch Test		+	-	+	-
	Fehlings Test		-	-	+	-
	Benedicts Test		-	-	+	-
Glycosides	Cardiac Glycoside & Phytosterols	Keller killiani Test	-	-	-	-
		Legal Test	-	-	-	-
		Libermann Test	+	+	+	-
		Baljet Test	+	+	+	+
	Anthra-quinone Glycoside	Brontragers Test	-	-	-	-
		Mod. Brontrager	-	-	-	-
	Saponin Glycoside		+	+	+	+
Tannins & Phenols	5% $FeCl_3$		+	+	+	+
	Gelatin Test		+	-	+	-
	Chlorogenic acid Test		-	-	-	-
	PPT. Test	Lead acetate	+	+	+	-
		Acetic acid	+	-	+	-
		Dil.HNO_3	+	+	+	-
		Pot. dichromate	-	-	-	-
Flavonoids	Zinc chloride Test		+	-	+	+
	Shinoda Test		-	-	-	+
	Lead acetate Test		+	-	+	+

Salacia reticulata **(SR),** *Pterocarpus marsupium* **(PM),** *Tinospora cordifolia* **(TC),** *Vetiver zizanioides* **(VR)**

Table 6. Physico-chemical Evaluation of *Salacia reticulate*

S. No.	Parameters	Value Obtained (mean ± SEM)	Standard Value (API)
1	Total Ash (%w/w)	2.36±0.14	Not yet reported
2	Acid insoluble Ash (%w/w)	0.82±0.26	Not yet reported
3	Alcohol soluble Extractive (%w/w)	6.62±1.16	Not yet reported
4	Water soluble extractive(%w/w)	5.28±0.32	Not yet reported

Table 7 Physico-chemical Evaluation of *Pterocarpus marsupium*

S. No.	Parameters	Value Obtained (mean ± SEM)	Standard Value (API)
1	Total Ash (%w/w)	1.86±0.24	Not more than 2 %
2	Acid insoluble Ash (%w/w)	0.22±0.10	Not more than 0.5 %
3	Alcohol soluble Extractive (%w/w)	10.32±1.26	Not less than 7 %
4	Water soluble extractive(%w/w)	9.88±0.30	Not less than 5 %

Table 8 Physico-chemical Evaluation of *Tinospora cordifolia*

S. No.	Parameters	Value Obtained (mean ± SEM)	Standard Value (API)
1	Total Ash (%w/w)	10.36±0.24	Not more than 16 %
2	Acid insoluble Ash (%w/w)	1.72±0.24	Not more than 3 %
3	Alcohol soluble Extractive (%w/w)	4.12±1.18	Not less than 3 %
4	Water soluble extractive(%w/w)	15.18±1.62	Not less than 11 %

Table 9 Physico-chemical Evaluation of *Vetiver zizanioides*

S. No.	Parameters	Value Obtained (mean ± SEM)	Standard Value (API)
1	Total Ash (%w/w)	7.87±0.57	Not more than 9 %
2	Acid insoluble Ash (%w/w)	5.82±0.52	Not more than 6 %
3	Alcohol soluble Extractive (%w/w)	6.34±1.23	Not less than 4 %
4	Water soluble extractive(%w/w)	5.89±0.22	Not less than 5 %

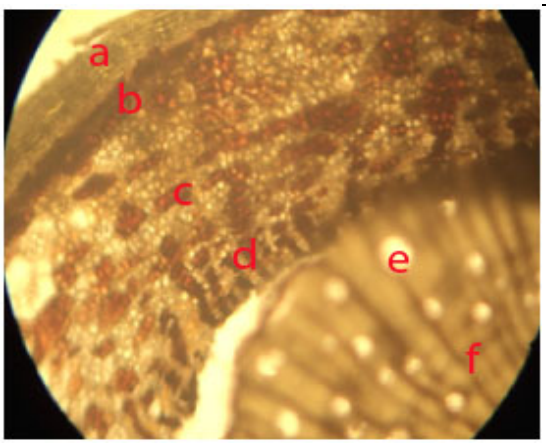

Fig. 1 Transverse section of *Salacia reticulata* root a) Outer cork b) Inner Cork c) Oil glannds d) Medullary rays e) & f) Xylem Vessels Fibres

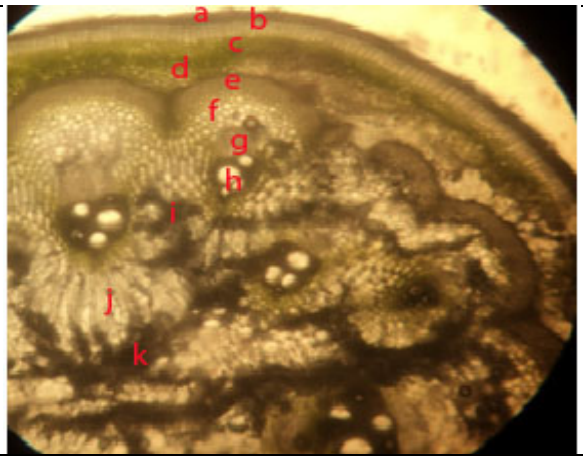

Fig. 2 Transverse section of *Tinospora cordifolia* stem a) Cork b) Collenchyma c) Parenchyma d) Medullary rays e) sclerenchyma f) Phloem g) xylem parenchyma h) Xylem vessels j) medullary rays k) Pith

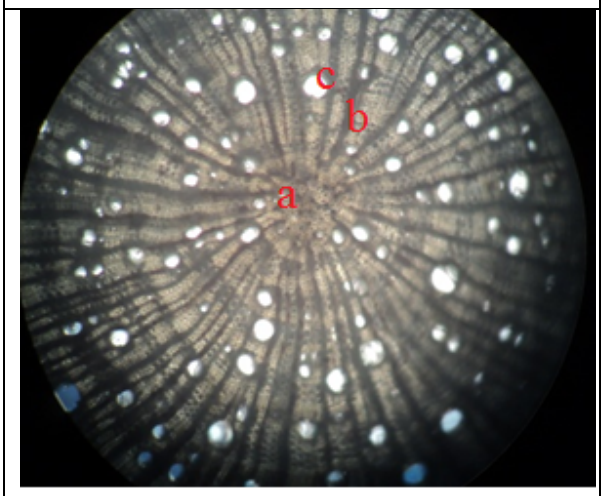

Fig. 3 Transverse section of *Pterocarpus marsupium* a) pith b) medullary rays c) vessels

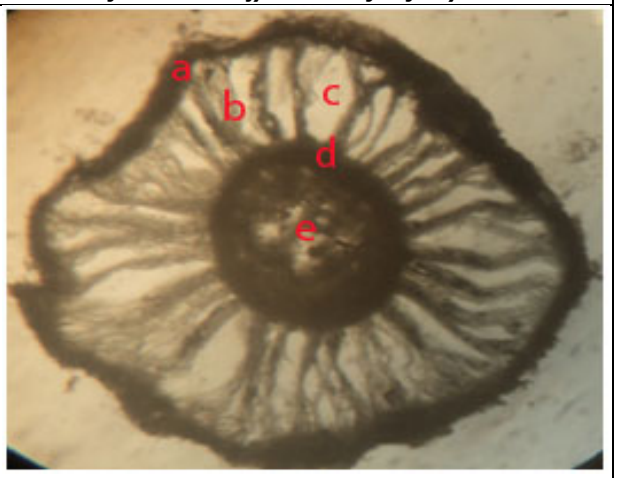

Fig. 4 Transverse section of *Vetiveria zizanioides* root a) Epidemis b) Hypodermis c) Aerenchyma strands d) Schlerenchymatous cortex e) Aerenchymatous pith

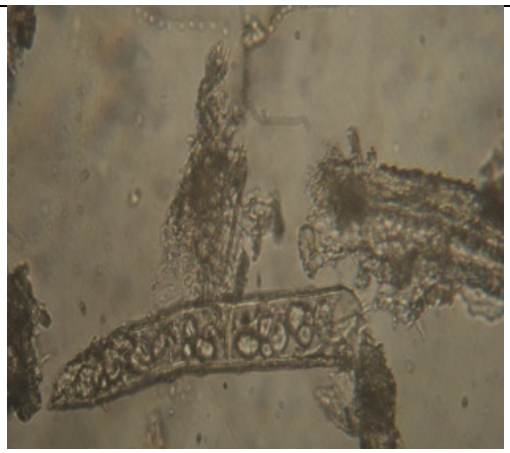

Fig. 5. Fibres with starch

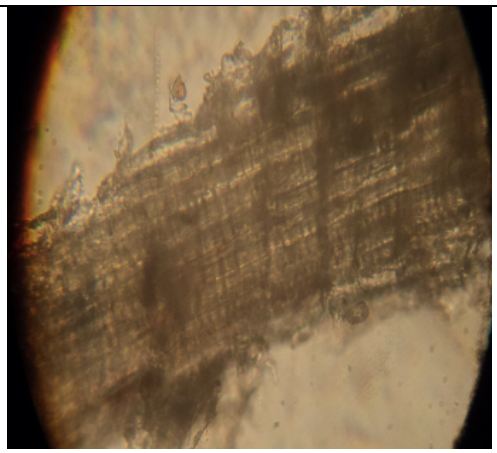

Fig. 6. Phloem fibres

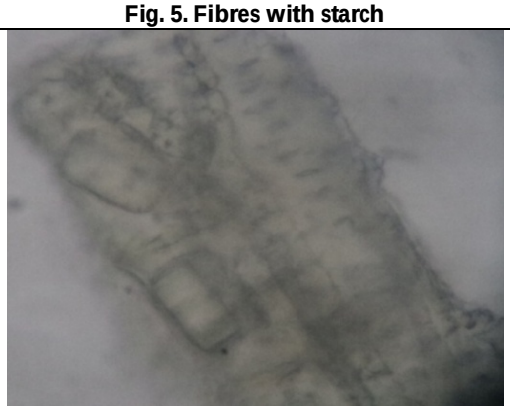

Fig. 7. Uniseriate medullary rays

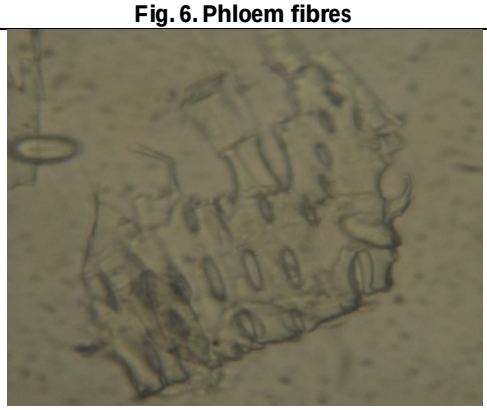

Fig. 8. Bordered pitted vessel

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RESEARCH ARTICLE

Molecular Interaction in Ternary Liquid Mixtures Containing N, N-Dimethylformamide, Butanol and Toluene at Different Temperatures

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ABSTRACT

The ternary mixtures of N, N-Dimethylformamide, Butanol and Toluene containing different ultrasonic properties have been studied at different temperatures and at a fixed frequency of 2 MHz. The ultrasonic related physical, chemical and thermodynamics parameters like velocity (U), density (ϱ), viscosity (η), adiabatic compressibility (β),intermolecular free length (L_f), free volume (V_f), relaxation time (τ), internal pressure (π_i), acoustic impedance (Z) and Gibb's free energy (G) are determined. The result is interpreted in terms of molecular interaction between components of mixtures.

Keywords: Ultrasonic velocity, N,N-dimethylformamide, thermodynamical parameters, adiabatic compressibility, free length, free volume, internal pressure, acoustic impedance.

INTRODUCTION

Intermolecular interaction between the component molecules of liquid mixtures consisting of polar and non-polar components is of considerable importance in understanding and they find various applications in several industrial and technological processes [1-3]. Moreover, the behaviour of a non-polar molecule in a different polar environment can also be discussed with the ternary system [4-6]. These liquid mixtures are of interest to organic chemists involved in the study molecular interactions. The values of ultrasonic velocity, density, viscosity and adiabatic compressibility as a function of concentration will be of much help in providing such information. Further, such studies as a function of concentration are useful in gaining insight into the structure and bonding of associated molecular complexes and other molecular processes [7-10]. The ultrasonic velocity measurement is a unique tool in characterizing the structure and properties of the liquid system and I provides significant information on the arrangement of matter in solutions and also finds an extensive application in studying the nature of intermolecular forces [11-14].

In the present paper, variation of various parameters of ternary mixtures containing n-Butanol,N,N-dimethylformamide(N,N-DMF)and at different temperatures have been studied for different concentrations. DMF (C_3H_7NO) is a polar solvent. The polar C=O. and C-N bonds make the molecule polar. DMF being a polar molecule results in dipolar and induced dipolar interaction between it and n-butanol in addition to dipolar-dipolar interaction between its molecules. DMF solutions are used to process polymer fibres, films and surface coating to permit easy spinning of acrylic fibre to produce wire enamels and as a crystallization medium in the pharmaceutical industry. N-Butanol (C_4H_9OH)is a primary alcohol having a linear structure. It is widely used as a solvent because many organic materials are soluble in it. Toluene is polar in nature due to the presence of electron releasing methyl group. Methyl group of toluene is an electron donor group through induction. Toluene thus interacts more strongly with DMF than with n-butanol.

EXPERIMENTAL SECTION

Various concentrations of the binary liquid mixtures were prepared in terms of mole fraction.
Solution-1 (S_1)→ DMF (1) + Butanol + Toluene:: 1 : 4 : 5
Solution-2 (S_2)→ DMF (2) + Butanol+ Toluene :: 2 : 4 : 4
Solution-3 (S_3)→ DMF (3) + Butanol+ Toluene :: 3 : 4 : 3
Solution-4 (S_4)→ DMF (4) + Butanol+ Toluene :: 4 : 4 : 2
Solution-5 (S_5)→ DMF (5) + Butanol+ Toluene :: 5 : 4 : 1
Solution-6 (S_6)→ DMF (6) + Butanol+ Toluene :: 6 : 4 : 0

Velocity Measurement

The ultrasonic velocity of sound wave in the ternary mixture have been measured using multi-frequency ultrasonic interferometer operating at 11 different frequencies (Model M-84) supplied by M/s Mittal Enterprises, New Delhi.

Density and Viscosity Measurement

The densities and viscosity of the liquid mixtures were measured using a digital meter named, "Micro-viscometer, Model-Lov is 2000 ME".

THEORETICAL ASPECT

Thermodynamic parameters were calculated b using the following relations [15-19].

Adiabatic Compressibility (β):

It can be calculated by using the equation of Newton Laplace [18] as,

$$\beta = 1/U^2.\varrho \text{ ---------- (1)}$$

Intermolecular free length (L_f):

It is calculated by using the relation[19],

$$L_f = K_T\beta^{1/2} \text{ ---------- (2)}$$

Where, K_T is the temperature dependent constant and 'β' is the adiabatic compressibility.

Free Volume (V_f)

Free volume liquid mixture can be calculated b using the relation [20]as follows

$$V_f = (M_{eff}.U/K.\eta)^{3/2} \text{ --------- (3)}$$

Where 'M_{eff}' is the effective mass of the mixture, 'K' is a dimensionless constant independent of temperature and liquid. Its value is 4.281×10^9[21].

Internal Pressure (π_i)
The internal pressure can be calculated by using the relation [22],

$\pi_i = bRT\,(k\eta/U)^{1/2}\,(\varrho^{2/3}/M^{7/6})$ ------- (4)

Where, the symbols have there usual meanings.

Relaxation time (τ)
Relaxation time is the time taken for the excitation energy to appear as translational energy and it depends on temperature and impurities. It can be calculated from the relation,

$\tau = 4/3.\,(\beta.\eta)$ ------- (5)

Acoustic impedance (Z)
The specific acoustic impedance is given by,

$Z = U.\varrho$ ------- (6)

Where, 'U' and 'ϱ' are velocity and density of the mixture.

Gibb's free energy
It can be calculated by using the relation

$\Delta G = kT.Ln\,(kT\tau/h)$ ------- (7)

Where, 'k' is the Boltzmann's constant and '*h*' is the Planck's constant.

Molar volume can be calculated by using the relation [23]

$Vm = M_{eff}/\varrho$ ---------- (8)

Available Volume
Schaffs et al [24] shown that the available volume can be obtained by the relation.

$Va = Vm(1-U/Um)$---------- (9)

Rao's Constant
R can be evaluated by an equation given by Bagchi et al [25]

$R = Vm.U^{1/3}$---------- (10)

Wada's Constant
Wada's constantis given by the relation [26]

$W = Vm.\beta^{-1/7}$ ---------- (11)

Surface Tension
Surface tension can be calculated by using the relation [27]
$S = 6.3 \times 10^{-4}.\varrho.U^{3/2}$---------- (12)

RESULTS AND DISCUSSION

The experimental data relating to density, viscosity and velocity at 288 K, 298 K, 308 K and 318 K for frequencies 2MHz for the mixtures are given in table-I. The calculated values of adiabatic compressibility (β), free length (L_f) and relaxation time (τ)are reported in table-2. Acoustic impedance (Z), Gibb's free energy (ΔG) and Internal pressure (π_i)are reported in table-3. Surface tenson (S), Rao's constant (R) and Wada's constant (W) are reported in table-4. Free volume (V_f), molar volume and available volume for the mixture are presented in table-5.The variation of different parameters with temperature are shown infig.1-4. The density values reported show that there isan increase in the density with an increase in the concentration of DMF. This maybe due to the molecular relaxation of DMF in Butanol and Toluene. The adiabatic compressibility values decrease with an increase in concentration of DMF. This trend of variation of adiabatic compressibility is inconsonance with the variation of ultrasonic velocity with concentration.

With increase in mole-fraction of DMF, Ultrasonic velocity increases. Also, velocity decreases with increase in temperature. This may be due to the structural changes occurring in the mixtures, which results in increase of intermolecular forces. Intermolecular free length is the distance between the surfaces of the neighbouring molecules in the mixture. Ultrasonic velocity in a solution depends on free length. As free length increases with temperature, ultrasonic velocity decreases with increase in temperature. Variation in free length indicates variation in molecular forces in the mixture which depends on the experimental density as well as temperature. It is found that free length decreases from S_1 to S_6 indicating increase in molecular interaction due to association in polar ends.

When the temperature is increases, molecules are move away from each other so there is a reduction in molecular interaction. This reduces the cohesive force. Thus, with increases in temperature, internal pressure decreases and free volume increases. In both the ternary mixtures, internal pressure decreases with increase in temperature indicating weakening of interaction with higher temperature. Contraction in volume leads to subsequent decrease in adiabatic compressibility as well as in intermolecular free length from S_1 to S_5 showing more cohesive forces as the concentration of n-butanol enhanced. These parameters increase with increase in temperature for a particular concentration as high temperature decreases the intermolecular force resulting in increase of volume.

Relaxation time is the time taken for the excitation energy to appear as translational energy. With increase in mole fraction of DMF, Relaxation time increase, which suggests that, the molecules get rearranged due to co-operation process. Relaxation time decreases with increases in temperature indicating the reverse process. As temperature increases excitation energy increases and hence relaxation time decreases. The decrease in the values of molar volume with increase in concentration of DMF indicates weak molecular interactions since the concentration change is very significant. Molar volume increases with rise in temperature. This is because thermal energy facilitates increase in molecular separation. Available volume is a measure of the compactness and strength of bonding between the molecules of the liquid mixture. As observed, it changes in the same way as molar volume. With increase in temperature, Gibbs' free energy increases, which suggests the closer approach of unlike molecule is due to hydrogen bonding [18-19]. The increase in Gibbs' free energy also suggests shorter time for rearrangement of the molecules in the mixtures. Decreasing trend of Gibbs free energy with increase in concentration of alcoholic group due to intra molecular hydrogen bonding and force of attraction between polar heads.

Acoustic impedance is the ratio of instantaneous excess pressure of any particle to the instantaneous velocity of that particle. Acoustic impedance decreases with increase in concentration of DMF as well as with increase in temperature. This indicates that there is a decrease in molecular concentration in both the cases. With the increase of temperature, Rao's and Wada's constant increases, which suggest the availability of a greater number of components in a given region indicating close packing of the medium. With increase in temperature, surface tension decreases

very slowly. This is also confirmed by the fact that the ultrasonic velocity increases slowly with temperature. This is because surface tension depends on density and velocity ($S \sim \varrho.U^{3/2}$).

CONCLUSION

The study of the viscosities, ultrasonic velocities, densities, and other parameters of N,N-DMF, n-butanol and toluene in various concentrations and temperatures reveals the characteristics and structural properties. It also indicates the presence of interactions between solute and solvent. These interactions also suggest the extent to which the bonding accelerates the physical and chemical properties. It is evident from the above study that the interactions of N.N-DMF and Toluene in n-butanol show tendencies of structure making of solute and solvent molecules together with solute-solute and solute-solvent interactions.

REFERENCES

1. Praharaj Manoj Kumar and Satapathy Abhiram, Indian Journal of Natural Sciences, 2021, 12(65), 29944-29949.
2. Praharaj Manoj Kumar, Satapathy Abhiram, Journal of Emerging technologies and innovative Research, 2019, 6(4), 351-353.
3. Praharaj Manoj Kumar and Sarmistha Mishra, Journal of Thermal Analysis and Calorimetry, 2018, 132(2), 1089-1094, ISSN: 1588-2926, DOI 10.1007/s10973-018-7038-9.
4. Praharaj Manoj Kumar and Mishra Sarmistha, International Journal of Interdisciplinary Research. and Innovations, 2018, 6(3), 272-278.
5. Praharaj Manoj Kumar and Satapathy Abhiram, Mishra Prativarani, Mishra Sarmistha, Journal of Theoretical and Applied Physics, 2013, 7(23).
6. Praharaj Manoj Kumar and Satapathy Abhiram, Indian Journal of Natural Sciences, 2020, 10(60), 19721-19725.
7. Paikaray R and Mishra S. Journal of Acoustic. Soc. of India, 2010, 37 (1), 20.
8. Praharaj Manoj Kumar, Satapathy Abhiram, Mohanty Jayashree, Mishra Sarmistha, International Journal of Engineering Research & Technology, 2014, 3(11), 1060-1065.
9. Praharaj Manoj Kumar & Mishra Sarmistha, International Research Journal of Engineering and Technology, 2020, 7(1), 950-955.
10. Panda S., and Praharaj M. K., Indian Journal of Natural Sciences, 2020, 10(59), 18552-18557.
11. Samantaray Biswajit, Praharaj Manoj Kumar and Das Smruti Prava, Journal of Ultra chemistry, 2021, 17(1), 1-8.
12. Panda S. and Mahapatra A. P., W. J. of Pharm. and Life Sc., 2018, 12, 76.
13. Praharaj Manoj Kumar, Satapathy Abhiram, Mishra Prativarani & Mishra Sarmistha, Journal of Chemical and Pharmaceutical Research, 2013, 5(1), 49-56.
14. Praharaj Manoj Ku. and Mishra Sarmistha, International Journal of Science and Research, 2015, special issue, 58-65.
15. Bagchi S., Nema S. K., and Singh R. P., Eur. Polym. J., 1986, 22, 859.
16. Wada Y., J. Phys. Soc. Japan, 1949, 4, 280. https://doi.org/10.1143/JPSJ.4.280
17. Batchinski A. J., Z Phys. Chem., 1913, 84, 643.
18. Vasantharani E. J., Kannagi K., Padmavathy R., IJP Appl. Phys., 2014, 52, 155.
19. Shelden R., Chem. Comm., 2001, 23, 2399. https://doi.org/10.1039/b10727

Table-1: Experimental vales of density, viscosity and ultrasonic velocity at different temperatures.

Sol.	Density (Kg/m^3)				Viscosity(η) NSm^{-2} (x 10^{-3})				Velocity (ms^{-1})			
	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K
S_1	0.8483	0.8394	0.8303	0.8210	0.8790	0.7510	0.6518	0.5705	1336.4	1323.0	1299.6	1283.7
S_2	0.8564	0.8476	0.8385	0.8293	0.9106	0.7822	0.6796	0.6016	1369.8	1327.5	1301.8	1273.7
S_3	0.8632	0.8543	0.8452	0.8362	0.9264	0.8063	0.7003	0.6185	1338.9	1322.1	1301.5	1287.7
S_4	0.8686	0.8598	0.8508	0.8417	0.9725	0.8365	0.7278	0.6413	1308.6	1294.2	1257.7	1234.6
S_5	0.8787	0.8698	0.8608	0.8517	1.0305	0.8868	0.7680	0.6776	1293.6	1281.8	1253.1	1222.8
S_6	0.8859	0.8770	0.8680	0.8587	1.0441	0.9026	0.7841	0.6896	1277.0	1257.5	1230.6	1205.0

Table-2: Calculated values of adiabatic compressibility, free length and relaxation time at different temperatures.

Sol.	Adiabatic compressibility x 10^{-10}				Relaxation time x 10^{-12}				Free length x 10^{-10}			
	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K
S_1	6.600	6.806	7.131	7.392	0.774	0.682	0.620	0.562	0.504	0.517	0.540	0.552
S_2	6.222	6.695	7.037	7.433	0.755	0.698	0.638	0.596	0.489	0.512	0.536	0.554
S_3	6.462	6.697	6.984	7.212	0.798	0.720	0.652	0.595	0.498	0.512	0.534	0.545
S_4	6.723	6.944	7.430	7.794	0.872	0.774	0.721	0.666	0.508	0.522	0.551	0.567
S_5	6.800	6.997	7.398	7.852	0.934	0.827	0.758	0.709	0.511	0.524	0.550	0.569
S_6	6.922	7.211	7.609	8.020	0.964	0.868	0.795	0.737	0.516	0.532	0.558	0.575

Table-3: Calculated values of acoustic impedance, Gibb's free energy and internal energy at different temperatures.

Sol.	Acoustic Impedance x 10^6				Gibb's free energy x 10^{-20}				Internal pressure			
	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K
S_1	1.134	1.111	1.079	1.054	1.160	1.196	1.234	1.271	1.606	1.533	1.479	1.426
S_2	1.173	1.125	1.092	1.056	1.159	1.197	1.235	1.273	1.625	1.572	1.519	1.481
S_3	1.156	1.129	1.100	1.077	1.161	1.198	1.236	1.273	1.667	1.608	1.550	1.501
S_4	1.137	1.113	1.070	1.039	1.164	1.201	1.240	1.278	1.735	1.663	1.615	1.568
S_5	1.137	1.115	1.079	1.041	1.167	1.204	1.242	1.281	1.810	1.733	1.675	1.632
S_6	1.131	1.103	1.068	1.035	1.168	1.206	1.244	1.283	1.844	1.775	1.717	1.668

Table-4: Calculated values of free volume, Rao's constant and Wada's constant at different temperatures.

Sol.	Surface tension				Rao's constant				Wada's constant			
	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K
S_1	26.11	25.45	24.51	23.79	243.3	245.1	246.3	248.1	168.7	169.8	170.5	171.5
S_2	27.36	25.83	24.81	23.75	243.0	243.0	244.0	244.9	168.5	168.5	169.1	169.7
S_3	26.64	25.87	25.00	24.34	239.3	240.8	242.1	243.8	166.3	167.2	168.0	169.0
S_4	25.91	25.22	23.91	23.00	236.0	237.5	237.8	238.8	164.3	165.3	165.4	166.0
S_5	25.76	25.15	24.06	22.94	232.4	234.0	234.7	235.3	162.2	163.2	163.6	163.9
S_6	25.47	24.64	23.60	22.63	229.5	230.7	231.4	232.2	160.5	161.1	161.6	162.1

Table-5: Calculated values of free volume, Molar volume and Available volume at different temperatures.

Sol.	Free volume x 10^{-7}				Molar volume				Available volume			
	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K	288 K	298 K	308 K	318 K
S_1	5.430	12.902	16.351	20.598	220.9	223.3	225.7	228.3	36.39	38.65	42.38	45.12
S_2	5.344	12.200	15.397	18.800	218.8	221.1	223.5	226.0	31.48	37.65	41.65	46.08
S_3	5.032	11.586	14.715	18.333	217.1	219.4	221.7	224.1	35.43	38.10	41.36	43.74
S_4	4.521	10.619	13.194	16.301	215.7	218.0	220.3	222.6	39.29	41.66	47.12	50.84
S_5	4.073	9.589	12.105	14.795	213.3	215.5	217.7	220.0	40.84	42.84	47.20	51.87
S_6	3.918	9.074	11.418	14.096	211.5	213.7	215.9	218.2	42.71	45.74	49.85	53.87

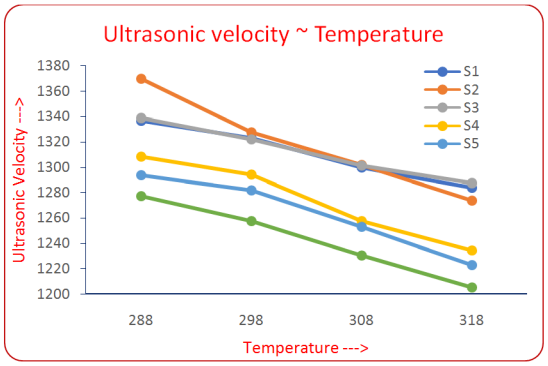

Fig-1: Variation of Ultrasonic velocity with temperature

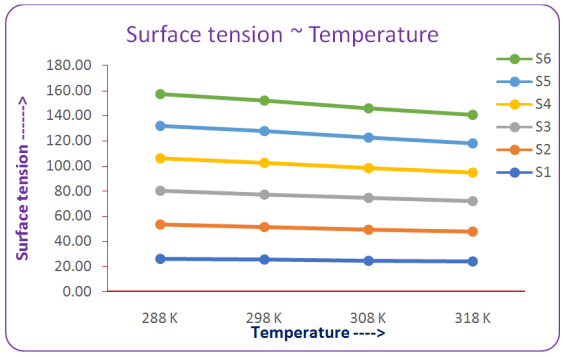

Fig-2: Variation of surface tension with temperature

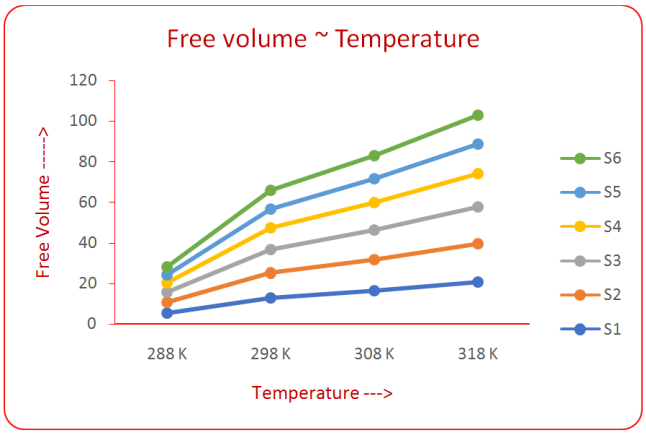

Fig-3: Variation of free volume with temperature

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RESEARCH ARTICLE

Heavy Metal Pollution and its Indexing Approach in Pre-monsoon Groundwater of Balangir and Puintala Blocks, Balangir District, Odisha, India

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ABSTRACT

The objective of this work is to find out the heavy metal pollution index of Balangir and Puintala blocks of Balangir District of Odisha during the pre-monsoon season of 2017. A total of 100 groundwater samples were collected to know the details about the concentration of heavy metal present in water. The atomic absorption spectrophotometer (AAS) is used to analyze heavy metals like iron, manganese, lead, chromium and cadmium. As per Bureau of Indian Standards (BIS), 2012, 13% of water samples were exceeded the acceptable limit for iron i.e. 300 µg/L whereas 22% of samples had lead content above the acceptable limit (10 µg/l). Only 3% samples of both chromium and cadmium has exceeded the acceptable limit where as only one sample is beyond the acceptable limit with respect to manganese. The mean HPI value of groundwater in the study area is 57.85. The heavy metal pollution index of 94% sample have value below 100 (critical index) and 6% have value above the critical level, which is due to agricultural, industrial and urban activity. The average MI value of the area of study is 0.54.

Keywords: Heavy Metal Pollution Index, Balangir district, Drinking Water Quality, Bureau of Indian Standards

INTRODUCTION

The area of investigation i.e. Balangir -Puintala block is situated in the western part of Odisha, which is drought-prone and consists of hard rock terrain. The demand for groundwater has increased due to industrialization and irrigation practices and population growth [Mahanta and Sahoo, 2012] [1]. For the sustenance of all living organisms, water is most essential. It is the key constituent of all living beings including plants, animals and other organisms for survival in the biosphere [Das and Mahanta, 2019] [2], [Muthulaksmi et al., 2009][3]. Now a day, heavy metals come under universal environmental contaminants. The pollution due to heavy metals in water creates a severe

environmental problem. Hence, a water contamination study is most important. The sources of heavy metals in groundwater is either anthropogenic or natural [Adaikpoh et al. 2005] [4], [Reza and Singh 2010] [5]. The concentration of metals and various minerals in the water is due to the weathering of minerals, rocks and soils of that area [Mahanta et al., 2020] [6] [Karbassi et al. 2008] [7]. Heavy metal contamination is mainly due to mining activities, disposal of effluents and metals from different industries, fertilizer and pesticides used in agricultural fields causes anthropogenic contamination [Zakhem and Hafez 2015] [8]. Heavy metal pollution index (HPI) and metal index (MI) are the most effective tools used for the estimation of heavy metals the pollution in groundwater.

STUDY AREA

The area of study is situated at Balangir District which comprises two blocks namely Balangir and Puintala and lies between longitude 83°13′15″E to 83°44′7″E and latitude 20°34′20″N to 20°51′8″N falling in Survey of India Toposheet numbers. 64P/1, 64P/2 64P/5, 64P/6, 64P/9 and 64P/10. It is surrounded on the north by Loisinga Block, south by Deogaon Block, west by Patnagarh Block of the same District and east by Sonepur District of Odisha. It is the hottest district in the western part of the state. Generally, the district experiences hot with low humidity from March to June.

Rock Type

The area belongs to the Easternghat Super group of rocks which consists of Khondalite, Charnockite, Gneissic rocks and Migmatites. The soil of the major part of the area is vertisols, which include medium black soils underlain by anorthosites. The soils of the area contain a high amount of potash, magnesium, calcium, iron and less in nitrogen and phosphorous. The texture is loam to clayey loam [Mahanta, 2017] [9], [Bakhara et al. 2019] [10]. The location map of the investigated area is given in Fig.1.

MATERIALS AND METHODOLOGY

To study the quality of groundwater in details, 100 numbers of water samples 2 from dug wells and 98 from tube well were collected in pre-monsoon, 2017. The samples were collected from the entire investigated area with more emphasis on the residential area, agricultural sectors, various industrial sites, etc. The sample location map is given in Fig.2. The various parameters physicochemical such as, , pH, temperature, total dissolved solids (TDS), electrical conductivity (EC), total alkalinity (TA), total hardness (TH), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), sulphate (SO_4^{2-}), chloride (Cl^{-}), carbonate(CO_3^{2-})), bicarbonate(HCO_3^{-}),nitrate(NO_3^{-}) , fluoride (F^{-})etc. were analyzed. Heavy metal concentrations like iron (Fe), manganese(Mn), lead(Pb), chromium(Cr) and cadmium(Cd) were also determined to know about the metal contamination in water by Atomic Absorption Spectrometer(AAS).

HEAVY METAL SPECIFICATION

Iron (Fe)

The iron concentration of the collected water varied from 14.6 to 361.7 µg/l with an averagee of 195.382µg/L. As per Bureau of Indian Standard (2012) [11], the acceptable limit of iron is 300 µg/l. The locations where the value exceeded the acceptable limit are Kudasingha (sample no.89), Patharla (sample no.94,95), Mahimunda (sample no.14), Bandanakata (sample no.83), Randa (sample no.77), Karlapita (sample no.33), Patharchepa (sample no.45), Kermeli (sample no.33), Chatamakhna (sample no.53), Lukapada (sample no.86) Sautpur (sample no.65) and Bhubel (sample no.31). The high concentration of iron is because of the discharge of waste effluents on land and weathering of rocks whereas the low value of iron indicates no major cause of pollution. The iron iso-concentration map of the area is given in Fig 3a and its bar diagram with sample location is given in Fig 3b Concerning BIS (2012), 13% of samples exceed the acceptable limit of iron for drinking purposes. The minimum, maximum, mean and standard deviation of the heavy metal concentration of the area of study is given in Table: 1. Manganese is found in form of various minerals and salts and is commonly found with iron compounds. The low concentration of manganese in groundwater is because of geochemical control [Jain et al. 2010] [12]. According to BIS, the acceptable limit of

manganese is 100 µg/L and the permissible limit is 300 µg/L. The concentration of manganese in groundwater samples ranged between1.16 and 105.6 µg/L with a mean of 32.11 µg/L All the samples were below the acceptable limit except for one which is found at Barkani village. The manganese concentration map is shown in Fig.4a and its graphical representation with sample site is given in Fig. 4b.

Lead (Pb)

Lead is used in the production of lead-acid storage batteries. It is also discharged from smelting, motor vehicle and corrosion of lead pipework [Gowd and Govil 2008] [13]. Lead is rarely present in groundwater because of its dissolution from natural sources. The lead concentrations of the study area ranged from 0.8 to 256 µg/L with a mean value of 11.04 µg/L. The 22% of water samples had exceeded their acceptable limit i.e.10 µg/L. Out of 22% samples, 20% of samples have values within 38 µg/l. Only two samples have exceeded the value of more than 100 which were seen in Bulusar having a Pb value of 112.8 µg/L (sample no.54) and Athgaon having a Pb value of 256 µg/L (sample no.. 100). The isoconcentration map of lead is shown in Fig. 5a and its graphical representation with sample site is given is in Fig. 5b.

Chromium (Cr)

The chromium value of the analyzed sample is ranged between 1.3 and 332.3 µg/L and average is 21.45µg/L. Analysis of samples collected from Balangir and Puintala blocks shows that all the samples were within the acceptable limit i.e. 50 µg/L for drinking water, except three numbers khaliapali,(sample No. 48,value115.6 µg/L), Sikachhida, (sample No. 18, value57µg/L), Sibtala,(sample no. 29, value51.3µg/L). The maximum concentration of chromium as 332µg/L was recorded at Bhaler (sample no. 98). The iso concentration map of chromium is given in Fig. 6a and graphical representation with sample site is given in Fig. 6b.

Cadmium (Cd)

Heavy metals like Cd, in drinking water, are the most toxic element even in very low concentration. In the human body, the biological half-life of Cd ranging from 10 to 33 years. The long time exposure to Cd induces disturbs the calcium metabolism in the body and also causes renal damage. Lungs and prostate cancer are also due to a high concentration of cadmium in water. The pollution of water is directly related to the contamination of water. Hence continuous monitor the quality of underground and surface water sources is needed. The maximum acceptable limit of Cd in drinking water is 3 µg/L as per BIS (2012). The Cadmium concentration of the area of study varied between 0.01 µg/L and 7 µg/l with a mean value of 1.29 µg/L. According to the BIS limit, 3% of the sample exceeded their acceptable limit for drinking purposes. The highest concentration of 7 µg/L was found in the village of Chudapali. The cadmium concentration map of the study area is given in Fig. 7a and its graphical representation with sample site is given in Fig. 7b. The comparison of heavy metals concentration with the BIS (2012) is given in Table: 2.

EXPERIMENTAL METHOD

Heavy Metal Pollution Index (HPI)

The method of rating which shows the composite influence of individual heavy metals on the overall quality of water is known as the Heavy Metal Pollution Index (HPI). [Sheykhi and Moore 2012] [14].

It is formulated by,

$$HPI = \frac{\sum_{i=1}^{n} W_i Q_i}{\sum_{i=1}^{n} W_i}$$

Where W_i = Unit weightage of i^{th} parameter

Q_i = Sub-index of the i^{th} parameter

n= Number of parameters

The sub-index (Qi) of the parameter is determined by the following formula.

$$Q_i = \sum_{i=1}^{n} \frac{|M_i - I_i|}{S_i - I_i} \times 100$$

where M_i= Monitored value of the heavy metals of the i^{th} parameter
I_i = Ideal value of i^{th} parameter
S_i = Standard value of the i^{th} parameter.

In the indexing of heavy metal Pollution, the rating value of Wi is an arbitrary value between 0 and 1 given for each metal. The rating is based on the relative importance of individual quality considerations and is inversely proportional to the recommended standard (Si) for each parameter. Water quality and its suitability for drinking may be examined by determining its quality index [Mohan *et al*. 1996] [15] [Prasad and Mondal 2008] [16]. The computational method for calculating HPI is given in Table- 3. The HPI values of 100 samples were calculated and the results were given in Table 4.

The critical pollution index of HPI value for drinking water is 100. Water quality can be classified into three categories based on HPI. These are low heavy metals pollution (<100), threshold risk (=100), high heavy metal pollution (>100) [Ghaderpoori et. al, 2018] [17]. It is found that only six numbers of groundwater samples have a high HPI value (>100). Most of the sample sites have a low value of HPI. The higher values of HPI were observed particularly in the area of Ramasingha (BT-34,), Chudapali (BT-47), Baidipali (BT-68). Sadeipali, (BT-74), Sautpur (BT-92), and Kurlupali (BT-100which may be due to the leaching of heavy metals from different industries of the study area. (Ref: Table 5).

Metal Index

The metal index is defined as evaluating the water quality based on the content of heavy metals present in water. If the metal concentration is higher than the MAC value, then the quality of the water is worse. The water cannot be used if the concentration of the element is higher than the respective MAC value (MI>1). The groundwater presence of several elements have smaller concentrations but close to the respective MAC values will also decrease the overall quality of water because of an additive effect. Thus, the metal index value 1 is the threshold of warning, even in the case where *Ci* is smaller than MAC*i* for all the elements. [Tamasi and Chini, 2004] [18].
MI can be express by the equation-

$$MI=\sum_{i=1}^{N} \frac{C_i}{(MAC)i} \qquad (3)$$

Where MI = metal index,
C = concentration of each element in solution,
MAC = maximum allowed concentration for each element
subscript i is the i^{th} sample. The computational method for calculating metal index (MI) for groundwater sample is shown in the table-6 and its concentration map is given in Fig.14.

The MI values of 100 samples were calculated and the results were given in Table 4. If the concentration of metals for MAC (Maximum Allowed Concentration) value is high, the water became worse quality. Therefore, the value of 1 for MI is a threshold of warning even in the case where Ci is less than MACi for all elements. (Where Ci is the mean concentration of each metal and MACi is maximum allowable concentration) (Balakrishnan and Ramu 2016) [19]. The average value of the metal index (MI) of the study area was found 0.54. The classification based on MI is given in Table: 7 which show that the village of Kurlupali (BT- 100) has MI value >100 and is strongly affected where as the village Ramasingha (BT-34) is moderately affected and Bhaler (BT-8) and Baidipali (BT-68) are affected slightly. About 86% samples are pure and 4% samples are very pure with respect to MI.

CONCLUSION

From the above studies, it is found that the average value of the heavy metal pollution index (HPI) of the area is 57.85 and metal index (MI) is 0.54. The HPI and MI values of the groundwater are used to evaluate the overall pollution level of groundwater. It is found that only six numbers of samples (6%) have a high HPI(>100), which is found in Ramasingha, Chudapali, Baidipali Sadeipali, Sautpur and Kurlupali. Similarly the only village Kurlupali is affected strongly by metal index and three locations i.e. Ramasingha, Bhaler and Baidipal are affected slightly.to moderate by metal index. As per Bureau of Indian Standards (BIS), 2021, 13% of water samples were exceeded the acceptable limit for iron i.e. 300 µg/L whereas 22% of samples had lead content above the acceptable limit (10 µg/l). Only 3% samples of both chromium and cadmium has exceeded the acceptable limit where as only one sample is beyond the acceptable limit with respect to manganese. The overall quality of groundwater is suitable for drinking purposes with respect to heavy metal pollution index except few locations.

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REFERENCES

1. Mahanta, N. and Sahoo, H.K.,(2012) .Remote sensing studies in delineating Hydrogeological parameters in the drought-prone Kuchinda-Bamra area in Sambalpur District, Odisha Int J Earth Sci Eng v.05(06),pp.1578-1583.
2. Das, A.K. and Mahanta, N.(2019) Evaluation of pre-monsoon groundwater quality oa Pallahara Block, Angul District, Odisha, India Int. Journ. of Emerging technologies and Innovative Research v.6 (5) pp.465-470.
3. Muthulakshmi, L., Ramu, A.and Kannan, N. (2009) Physico – chemical characteristics of ground water in and around Sivakasi region, Indian J. Environ. Protec. 29(5), 435-438.
4. Adaikpoh, E. O., Nwajei, G. E and Ogala, J. E. (2005). Heavy metals concentrations in coal and sediments from river Ekulu in Enugu, Coal City of Nigeria. J. Applied Sciences and Environmental Managementv. v.9(3),pp.5-8.
5. Reza, R.and Singh, G. (2010) Assessment of heavy metal contamination and its indexing approach for river water. Int J Environ Sci Tech v.7(4),pp.785–792
6. Mahanta N., Mishra I., Hatui A., Mahanta P.S.; Sahoo H.K., Goswami S. (2020) Geochemical appraisal of groundwater qualities and its uses in and around Maneswar Block of Sambalpur District, Odisha, India Environ Earth Sci 79:5 https://doi.org/10.1007/s12665-019-8719-9
7. Karbassi, A.R., Monavari, S.M., Nabi Bidhendi GR, Nouri J and Nematpour K (2008) Metal pollution assessment of sediment and water in the Shur River. Environ Monit Assess v.147(13),pp.107–116
8. Zakhem, B.A.and Hafez, R.(2015) Heavy metal pollution index for groundwater quality assessment in Damascus Oasis, Syria, Environ Earth Sci v.73,pp.6591–6600.
9. Mahanta, N (2017) Groundwater Investigation by Resistivity Method in the Drought –Prone Kuchinda and Bamra Blocks, Sambalpur District, Odisha, India Int J Earth Sci Eng v.10(02),pp.242-246 DOI: 10.21276/ijese.2017.10.0215
10. Bakhara,B., Mahanta,N.and Sahoo, H.K.(2019) Interpretation of groundwater chemistry using Piper and Chadha's diagrams: a comparative study from Balangir and Puintala blocks of Balangir district, Odisha, India, JETIR, v. 6, pp.35-38.
11. BIS (Bureau of Indian Standards) (2012) Specification for drinking water IS 10500: 2012, New Delhi, India.
12. Jain, C.K., Bandyopadhyay, A.and Bhadra, A. (2010) Assessment of ground water quality for drinking purpose, District Nainital, Uttarakhand, India. Environment Monitoring Assessment, v.166, pp. 663-676.

13. Gowd, S.S. and Govil, P.K. (2008) Distribution of heavy metals in surface water of Ranipet industrial area in Tamil Nadu, India. Environ Monit Assess 136:197-207.
14. Sheykhi, V.and Moore, F. (2012) Geochemical characterization of Kor River water quality, Fars Province, Southwest Iran. Water Qual Expo Health v.4, pp.25–38
15. Mohan, S.V., Nithila, P.and Reddy, S.J. (1996) Estimation of heavy metal in drinking water and development of heavy metal pollution index. J Environ Sci Health A v.31(2),pp.283–289
16. Prasad, B.and Mondal, K.K. (2008) The impact of filling an abandoned opencast mine with fly ash on ground water quality: a case study. Mine Water Environ v.27(1),pp.40–45
17. Ghaderpoori, M., kamarehie,B., Jafari, A., Ghaderpoury, A., and Karami, M.,(2018) Heavy etals analysis and quality assessment in drinking water – Khorramabad city, Iran, Elsvier, doi: 10.1016/j.dib.2017.11.078**,** pp-685-692.
18. Tamasi, G. and Cini, R. (2004) Heavy metals in drinking waters from Mount Amiata (Tuscany, Italy) Possible risks from arsenic for public health in the province of Siena, Sci. Total Environ. V.327 ,pp.41-51.
19. Balakrishnan,A. and Ramu, A. (2016) evaluation of heavy metal pollution index(HPI) of ground water in and around the coastal area of gulf of mannar biosphere and palk strait J. Adv. Chem. Sci. v.2(3),pp. 331–333.

Table 1: Statistical data of heavy metal of water samples of the area of study

Parameter	Minimum	Maximum	Average	SD
Fe	14.6	361.7	195.38	82.78
Mn	1.16	105.6	32.11	20.66
Pb	0.8	256	11.04	27.35
Cr	1.3	332.3	21.45	34.86
Cd	0.1	7	1.29	1.04

Here, all the values are expressed in µg/L.

Table 2: Comparison of Chemical quality of groundwater with BIS for drinking and domestic purposes

Parameters	BIS 10500:2012		No. of Samples exceeding acceptable limits	% of samples exceeding acceptable limit	No. of Samples exceeding Permissible Limit	% of samples exceeding Permissible Limit
	Acceptable Limit	Permissible Limit				
pH	6.5-8.5	No relaxation	27	27	Nil	0
TDS	500	2000	61	61	1	1
TH	200	600	84	84	5	5
Ca^{2+}	75	200	43	43	3	3
Mg^{2+}	30	100	40	40	1	1
Cl^{-}	250	1000	3	3	Nil	0
SO_4^{2-}	200	400	1	1	Nil	0
NO_3^{-}	45	No relaxation	23	23	Nil	0
Fe^{+}	0.3	No relaxation	13	13	Nil	0
Mn^{4+}	0.1	0.3	1	1	Nil	0
Cr^{3+}	0.05	No relaxation	3	3	Nil	0
Cd^{2+}	0.003	No relaxation	3	3	Nil	0
Pb^{2+}	0.01	No relaxation	22	22	Nil	0

All units are in µg/l., except TDS (mg/L) and pH.

Table 3: Calculation of Heavy Metal Pollution Index (HPI) for groundwater samples

Heavy metals	Mean Value(Mi) in ppb	Standard Permissible Value(Si)in ppb	Highest Desirable Value (Ii) in ppb	Unit Weightage (Wi)	Sub Index (Qi)	Wi×Qi
Fe	195.38	300	--	0.003	65.13	0.20
Mn	32.11	100	300	0.003	33.95	0.10
Pb	11.04	10	--	0.1	110.4	11.04
Cd	1.29	3	--	0.33	43	14.19
Cr	21.45	50	--	0.02	42.9	0.86
				$\sum W_i$=0.456		$\sum W_i Q_i$ =26.39

Heavy metal pollution index (HPI) =26.39/0.456=57.87.

Table 4: HPI and MI values of groundwater in study area.

Sample. No	HPI	MI	S.No	HPI	MI	Sample. No	HPI	MI	S.No	HPI	MI
1	40.72	0.40	26	54.12	0.45	51	44.88	0.46	76	43.13	0.44
2	28.01	0.31	27	39.10	0.39	52	95.55	0.64	77	34.62	0.33
3	49.55	0.46	28	22.47	0.46	53	80.50	0.57	78	46.94	0.30
4	24.49	0.24	29	30.10	0.39	54	72.09	0.54	79	41.74	0.38
5	31.94	0.30	30	18.65	0.32	55	27.39	0.33	80	60.77	0.30
6	25.83	0.31	31	22.39	0.33	56	44.87	0.41	81	38.04	0.36
7	31.31	0.30	32	28.01	0.29	57	85.51	0.48	82	26.53	0.36
8	79.11	1.73	33	99.07	0.69	58	55.10	0.68	83	68.20	0.59
9	73.99	0.53	34	265.07	2.57	59	49.30	0.37	84	62.33	0.52
10	34.38	0.36	35	31.80	0.50	60	20.95	0.20	85	19.69	0.44
11	33.80	0.46	36	61.62	0.54	61	69.15	0.49	86	54.60	0.39
12	60.17	0.59	37	43.69	0.39	62	51.84	0.55	87	72.10	0.44
13	44.47	0.37	38	60.02	0.38	63	36.94	0.38	88	39.82	0.39
14	41.55	0.48	39	41.05	0.36	64	22.13	0.37	89	29.58	0.41
15	34.71	0.57	40	32.11	0.49	65	54.76	0.45	90	31.55	0.36
16	24.59	0.29	41	45.77	0.56	66	30.55	0.30	91	19.70	0.38
17	42.90	0.38	42	66.57	0.33	67	32.87	0.28	92	102.63	0.81
18	55.07	0.81	43	86.93	0.56	68	132.51	1.09	93	46.90	0.56
19	18.67	0.36	44	65.75	0.46	69	34.02	0.34	94	62.58	0.37
20	35.92	0.33	45	31.97	0.41	70	32.44	0.28	95	80.91	0.53
21	82.09	0.65	46	45.84	0.48	71	67.68	0.63	96	28.82	0.34
22	53.81	0.40	47	204.43	0.86	72	34.01	0.55	97	44.80	0.37
23	29.34	0.49	48	72.48	0.50	73	34.89	0.40	98	70.54	0.46
24	31.93	0.30	49	27.22	0.35	74	174.02	0.84	99	36.02	0.31
25	43.88	0.45	50	23.04	0.30	75	64.76	0.54	100	601.99	5.48

Table -5: Water quality classification using HPI (Ghaderpoori et. al, 2018)

HPI	Characteristics	% of samples
<100	Low heavy metals pollution	94
=100	Threshold risk	0
>100	High heavy metals pollution	6

Table 6: Calculation of Metal Index (MI) for groundwater sample

Heavy Metals	Concentration(C_i) in (ppb)	Maximum Allowable Concentration. (MAC)	MI=$\sum_{i=1}^{N} \frac{C_i}{(MAC)\,i}$
Fe	195.38	300	0.65
Mn	32.11	300	0.11
Pb	11.04	10	1.1
Cd	1.29	3	0.43
Cr	21.45	50	0.43
			Mean =0.54

Table 7: Water quality classification using MI (Caerio et al. 2005, Lyulko et al., 2001)

MI	Characteristics	Class	Sampling no.	Total no. of Sample
<0.3	Very pure	I	4, 60,67,70	03
0.3-1.0	Pure	II	All the samples except class I, III, IV and V.	93
1.0-2.0	Slightly affected	III	8,68	02
2.0-4.0	Moderately affected	IV	34	01
4.0-6.0	Strongly affected	V	100	01
>6.0	Seriously affected	VI	---	

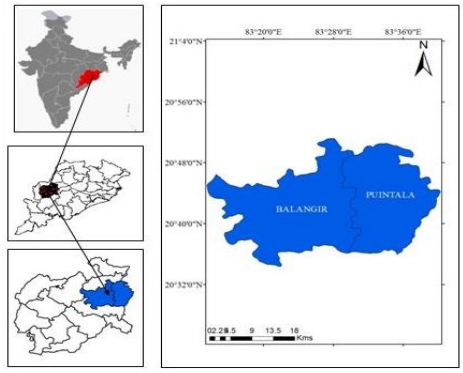

Fig. 1: Location map of study area

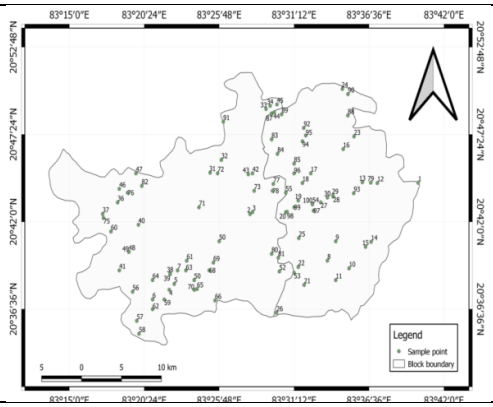

Fig.2: Sample location map

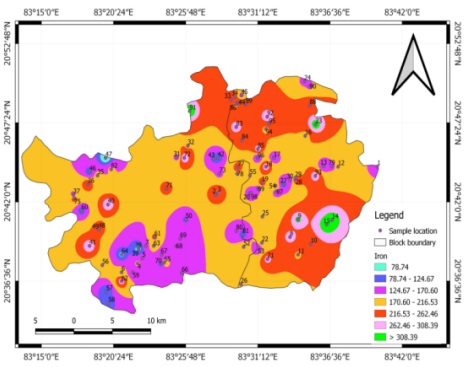

Fig.3a: Spatial distribution map of Iron

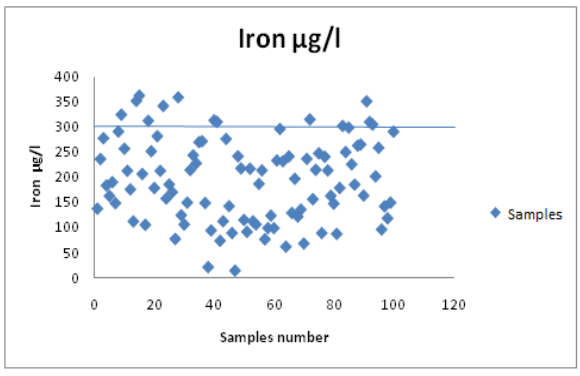

Fig .3b :Concentration map of Iron

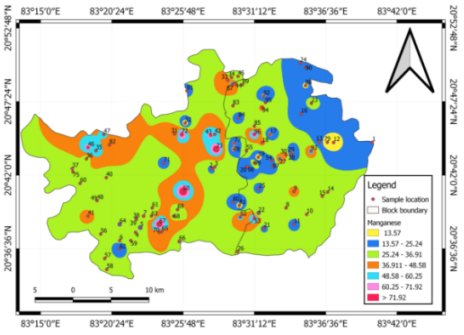

Fig.4a: Spatial distribution map of Manganese

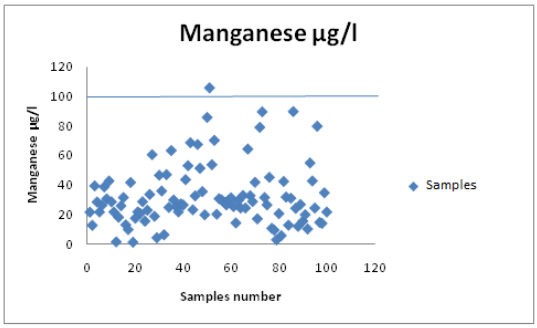

Fig .4b: Concentration map of Manganese

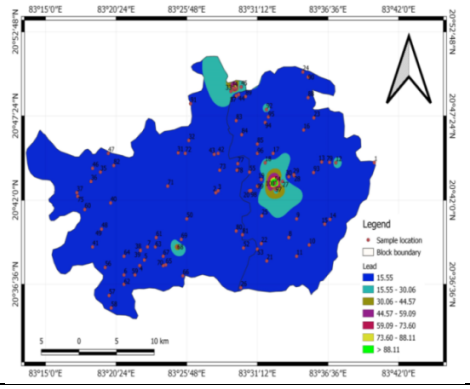

Fig .5a: Spatial distribution map of Lead

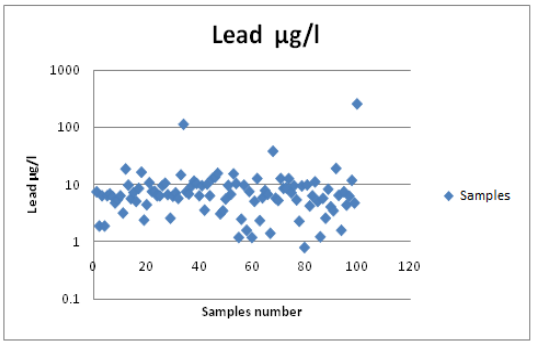

Fig .5b: Concentration map of Lead

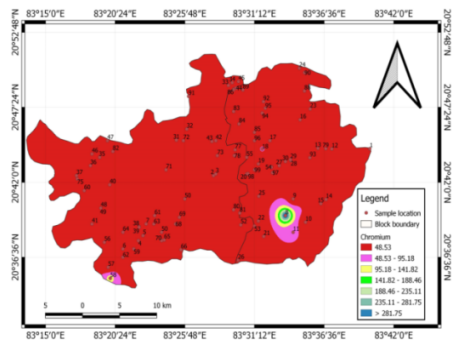

Fig .6a: Spatial distribution map of Chromium

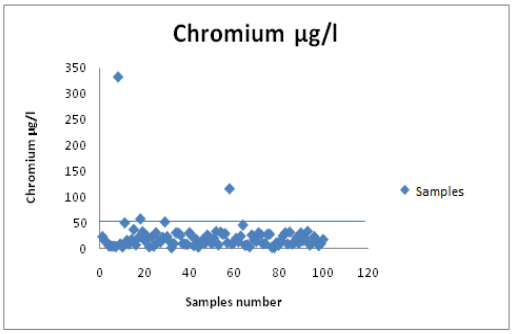

Fig .6b: Concentration map of Chromium

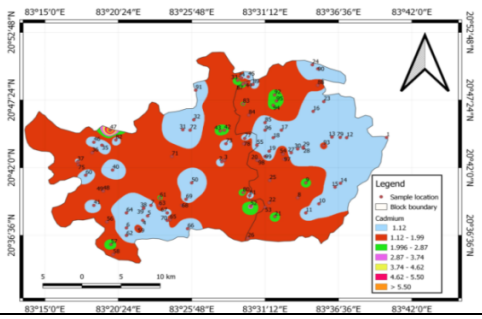

Fig .7a: Spatial distribution map of Cadmium

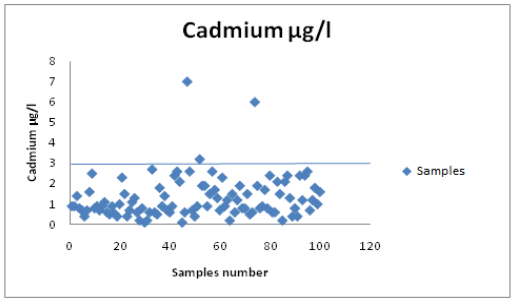

Fig .7b: Concentration map of Cadmium

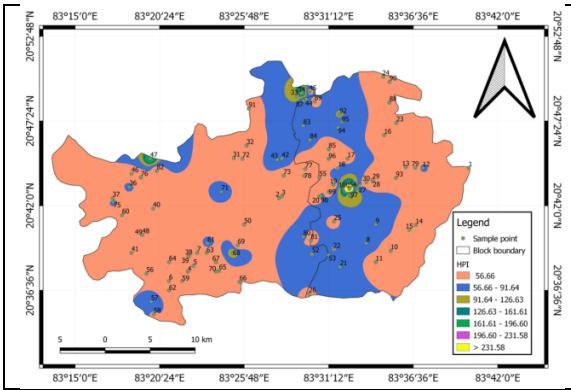

Fig .8: Spatial distribution map of Heavy Metal Pollution Index (HPI)

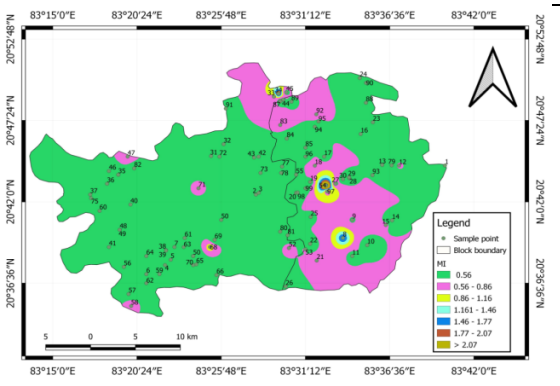

Fig .9: Spatial distribution map of Metal Index (MI)

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RESEARCH ARTICLE

In vitro Dissolution of Some Kidney Stones by Aqueous Extracts of *Paronychia argentea* L. and *Paronychia capitata* L. (Antilitholytic Activity)

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ABSTRACT

The limitations of drug therapies in the treatment of lithiasis have led researchers to discover other alternative therapies in traditional medicine such as the use of plants or their extracts. In this study, the effect *in vitro* of aqueous extracts of two plants *Paronychia argentea L.* and *Paronychia capitata L.* on the dissolution of kidney stones was evaluated. Our work was based on kidney stones weighing from 1.174 ± 5 g to 3.445 ± 5 g that examined their morpho-constitutional analysis by qualitative chemical and IR spectroscopic analysis who are placed in contact with the aqueous extracts of both plants during a period of 8 weeks, after which different dosages were carried out: the weight of the stones, the pH of the dissolving tea, the rate of dissolution (%) and the chemical analysis of some ions such as Calcium, Magnesium and Phosphorus, as well as the characterization of the saponoside contents in the aqueous extracts of two plants. The results show that the plant *P. argentea* contain a high level on saponoside than *P. capitata*. *Paronychia argentea L.* has a more significant dissolution effect than that of *Paronychia capitata L.*, especially on mineralo-organic (anhydrous uric acid) type stones. *Paronychia argentea L.* exhibits a significant 40% dissolution effect on anhydrous uric acid kidney stones compared to calcium oxalate type

stones. While *Paronychia capitata L.* shows no significant dissolution effect with respect to this type of kidney stone, this is undoubtedly due to the high levels of saponoside of the type of oleanane it contains compared to those contained in *Paronychia capitata L.* and which gives it this higher anti-lithiasis power.The in vitro dissolution effect of both plants is probably related to the saponoside content of the oleanane type they contain. At the limits of our experience, we cannot recommend these plants for the curative and / or prophylactic treatment of kidney stones. Long-term additional *in vitro* with multiple repetitions of each kidney stone type and *in vivo* tests are required to demonstrate some of the curative and preventive effects of the extracts tested on urinary stones. Therefore, complementary testing is needed for the confirmation of their anti-lithiasis effect and the active compounds responsible for this activity.

Keywords: Kidney stone, Aqueous extracts, *Paronychia argentea L.*, *Paronychia capitata L.*, *In vitro*, Anhydrous uric acid, Calcium oxalate, Saponoside.

INTRODUCTION

Urinary lithiasis is a frequent multifactorial pathology that affects, depending on the country, 4 to 20% of the population[1][2]. Despite the progress in pharmacology, the therapeutic use of medicinal plants is very present in some countries of the world and especially in developing countries such as Algeria, particularly in the absence of a modern medical system[3][4]. Urinary lithiasis can have a wide variety of causes: metabolic, nutritional, infectious, anatomical or drug-related and whose identification requires clinical and biological investigations[5]. The physicochemical analysis of urinary stones provides information that can effectively contribute to the understanding of the mechanisms involved in their formation. The identification of the causes of lithiasis allows the implementation of effective therapeutic or dietary measures leading to the reduction or cessation of recurrent calculi[6][7][8]**.** Algeria has a very rich plant biodiversity, traditional medicine is still widely used and herbal medicine is favorably received by the population because of its sensitivity to ethnic traditions[9]. The use of medicinal plants is self-medicated and based on recommendations based on each other's experience. The use of non-conventional medicine, in particular phytotherapy, is generally due to the failure of conventional treatment or the limited adverse effects of conventional drugs used in this case in the treatment of certain lithiasis[10]**.** The objective of this study is to characterize the *in vitro* dissolution capacity of aqueous extracts of both plants: *Paronychia argentea L.* and *Paronychia capitata L.* and to highlight the relationship between the amount of saponosides they contain and the antilithiasic effect.

MATERIALS AND METHODS

On the one hand, we extracted the active ingredients (secondary metabolites) present in the aqueous extracts of the two plant species studied, namely: *Paronychia argentea L.*,*Paronychia capitata L.*, with the dosage qualitative of saponoside contents. In addition, the effect of infusion teas prepared from these two plants on the in vitro dissolution of a few kidney stones the different weights and morpho-constitutional types for eight weeks was studied. This effect is controlled by measuring the following parameters:

- A morpho-constitutional analysis of the kidney stone by qualitative chemical and IR spectroscopic techniques.
- The weight of the calculi put in the dissolving herbal teas, the weight loss and the dissolution rate (%).
- The pH of the dissolving herbal teas.
- A chemical analysis of some ions (Calcium, Magnesium, Phosphorus) present in dissolving herbal teas.

Kidney stones

In the dissolution test, kidney stones of different morphological aspects were used((a) kidney stone of the anhydrous uric acid type; (b) et (d)kidney stone of the calcium oxalate monohydrate type; (c) kidney stone of the calcium phosphate type)that Obtained from the Department of Urology, Department of Nephrology, Benaouda Benzerdjeb - Oran- University Hospital (CHU-Oran- Algeria). These calculi were washed and stored in physiological water. Before the dissolution test, the fragments of kidney stones are washed with distilled water and then dried in the open air on filter paper for 16 hours at a temperature of 40°C. The morphological, dimensional and weight parameters are then measured.

Qualitative analysis by binocular microscope examination for morpho-constitutional determination of urinary calculi and Fourier Transform Infrared Spectrophotometry (FTIR) analysis in order to determine of chemical and crystal composition. An IR absorption spectrum is recorded following exposure of the sample to infrared radiation The location of absorption peaks is specific to the chemicals present and their height is proportional to their quantity. To simplify the analysis of the absorption spectrum obtained, it is compared with a reference spectrum bank using software. This bank was created at the SUHC (Sherbrooke University Hospital Center) and contains approximately 400 spectra from specimens, pure chemicals and mixtures of products whose concentrations are known. The software selects five spectra similar to the one obtained and the staff makes the final identification[11]**.**

The determination of the molecular and crystalline composition of kidney stones

For IR analysis all stones were dehydrated overnight at 37°C, then binocularly viewed (morphological) and fully pulverized (layer-by-layer when the computation exhibits a distinct stratified appearance) in a mortar agate. A small fraction of this powder (about 1 mg) is mixed in an agate mortar with about 100 mg of potassium bromide (KBr) (substance not absorbing IR radiation in the radiation zone studied) which has been previously dried up one night at 100 ^{o}C. A fraction of the mixture is then molded into pellet 13 mm in diameter under a manual hydraulic pressure of 10 tons. The pellet thus prepared is analyzed by an IR spectrophotometer (I.F.S. 100 bruker) which allows a direct indexing of the peaks over a spectral range in the infra-red medium spreading from 4000 to 500 cm^{-1}[12][13].

Plant material and extraction

Paronychia is a genus that belongs to the subfamily of Paronychioideae. It is represented by five species in the flora of Algeria. *Paronychia capitata L*. known under the name of (*Atai el Djebel*). The infusion of the aerial parts of the plant has been used in Spanish folk medicine to purify blood, regulate the circulation and treat gout, as well as an agent for dermatitis and as an expectorant. It is also used as cholagogue, dermatologic, anti-infective, lithotritic, diuretic, digestive and antihypertensive. Although there are no reports of the medicinal uses of *P. capitata*, the aerial parts of *P. argenteaLam*. are used in the Algerian popular medicine for the treatment of renal diseases, diabetes, and as diuretic[14][15]. Both plants belong to the family Caryophyllaceae of the genus *Paronychia* (*P. argentea L.,* and *P. capitata L*.) They were harvested during the flowering period from April to June 2017 in the forest of the Mountain Lions (stony grazing) located east of the city of Oran- in Algeria. The region is characterized by the following geographic coordinates: Altitude: 35° 45'24.91 '' to the North, Longitude: 0° 30'02.5"to West. Altitude in relation to sea level is 299 m.

The identification of these two plants was done by Prof. Hadjadj A. S. & Dr. Bahi K., in the herbarium of the laboratory of plant ecology, Oran University 1 - Ahmed Ben Bella - Algeria. The aerial parts were dried in an airy place in a dark place for about fifteen days, while the roots were removed, sieved to remove soil and debris and cut into small pieces and stored in well closed paper bags. For each plant, an aqueous extract is prepared by decoction for 30 minutes by adding 12 g of vegetable powder to 600 ml of boiling aqueous sodium chloride solution (NaCl at 9 g/l), its concentration of dissolved elements is comparable to that of the main biological fluids present in the body such as blood plasma. Careful agitation is used to facilitate the diffusion of the active ingredients. After 60 minutes, each extract is filtered through a filter attached to a 100 ml filtration bottle using a funnel to obtain the decocted tea[16].

Characterization of saponosides

Saponosides are very common substances in plants. They are characterized by their foaming power in aqueous solution which gives the foam index. The characterization of saponosides by the foam indexis done according to the method described by Badiaga M., 2011[17].

Evaluation of litholytic activity i*n vitro*

In vitro dissolution is carried out on the basis of the method described by Hannache B. et al. 2012[18]. The aqueous extract of each prepared plant was distributed in three Erlenmeyer flasks, each containing 50 ml for the aqueous extract of *Paronychia argentea* and the other three for the aqueous extract of *Paronychia capitata L*. The fragments of each kidney stone are placed in a porous braided fiber bag to avoid contact with the magnetic bar and then immersed in the dissolving tea. The effect of the extracts from these two plants was compared to that of a control aqueous extract from each plant.Each extract was put under helical magnetic agitation at 3000 rpm for one week. After incubation, the calculi are collected in filter paper and washed in distilled water. At the end of each week, the fragments of each washed kidney stone are placed in a glass box and dried at 40°C for 16 hours, after complete drying their weight is measured using a 100th milligram precision balance (Sartorius) to assess mass loss, then the pH of the herbal teas is measured and colorimetric measurements are made of a few ions (Calcium, magnesium, phosphorus). After the evaluation and dissolution tests were completed, the kidney stones were placed in the prepared extract for another week; the tests were performed weekly for a period of two months. Each of the experiments was repeated three times under the same conditions, and the results were expressed by calculating the mean ± standard deviation of the values obtained[19].

Evaluation of the ability of aqueous extracts of plants to dissolve kidney stones

The dissolution activity of the kidney stones by each extract was evaluated by calculating the dissolution rate of the kidney stones after stay in the experimental medium, according to the residual weight of the stones compared to their initial weights before the incubation in the new prepared extract [20]. The percent dissolution was calculated by the following formula:

$$A\% = (W_{initial} - W_{final}) \times 100 / W_{initial}$$

A% is the dissolution rate of a kidney stone.

$W_{Initial}$ and W_{final} are respectively the weights of the kidney stone before and after the incubation in the extract of each plant.

Evaluation of the pH of in vitro dissolution tea

The pH of the dissolving herbal teas is measured by a pH meter (Combo pH) which is based on the reading of the pH value of a solution tested by the introduction of an electrode sensitive to hydronium concentration; the value is displayed digitally by the light-emitting diodes. The initial pH of the solutions was measured, then it is measured weekly throughout the experimental period.

Spectrophotometric determination of ions concentration (Calcium, Magnesium, phosphate)

Calcium concentration

Calcium is measured by the O-Cresophthalein V/V colorimetric method. The measurement of the calcium concentration in a solution is based on the formation of a colored complex between calcium and O-Cresophthalein through an alkaline solution. The intensity of the color formed is proportional to the concentration of calcium in the solution [21].

$[Ca^{+2}]$ mg/ml = [(A) Sample / (A) Standard] x10 (**A**): Absorbance at 570 nm.

Magnesium concentration

Magnesium is determined by the Xylidyl blue colorimetric method. Magnesium reacts with Magon sulfonate in alkaline solution to form a colored complex. The intensity of the color formed is proportional to the concentration of magnesium in the solution[22].

[Mg^{+2}] mg/ml = [(A) Sample / (A) Standard] x 2 (**A**): Absorbance at 546 nm.

Phosphorus concentration

The phosphorus is measuredby colorimetric phosphomolybdate method. Inorganic phosphorus reacts with molybdic acid to form a phosphomolybdic complex. The reduction in alkaline medium allows the development of a blue color of molydenium. The intensity of the color formed is proportional to the concentration of inorganic phosphorus in the solution[23].

[P^{+3}] mg / ml = [(A) Sample / (A) Standard] x 5 (**A**): Absorbance at 710 nm.

Theoretical evaluation of the phenomenon of dissolution of kidney stones *in vitro*

Theoretical evaluation by *in silico* methods are complementary to *in vivo* and *in vitro* studies on living phenomena and cannot replace experience; they provide information on the nature of the chemical interactions between active molecules of medical interest and their action on target receptors or molecules and are necessary for the proper understanding of these mechanisms of action. In other words, the combined use of theory and experience leads to a better understanding of the phenomena dealt with in a field of research [24]. For this, we generally use graphical interface software and molecular modeling by molecular mechanic's methods such as *Accelrys 6.0* software in order to understand how the interactions (hydrogen bonds) are effected between these two molecules which generate the formation of dissolution complex.

RESULTS AND DISCUSSION

Results relating to the analysis of urinary stones

The morphological characteristics of kidney stones collected after surgery (by percutaneous nephrolithotomy) are shown in Table 1. The results of optical examinations and morphological typing of the tested kidney stones are shown in Table 2. The results of the qualitative analysis tests of the chemical composition of these kidney stones are shown in Table 3.

The FT-IR (ATR Diamond) analysis results of these kidney stones

The results of qualitative and spectral IR chemical analysis define that these kidney stones have different compositions including mineral or organic compounds. According to the reference of FTIR spectroscopic study the human urinary stones and table of type the occurrence and IR bands of principle components observed in kidney stones[25][26].The spectrum IR of the kidney stone (a) defines that is the type IIIa + type Ia (anhydrous uric acid (mostly) + calcium oxalate). The Spectrum IR of the kidney stone (b) and (d) defines that are the type Ia (Whewellite) + type Iva = Carbapatite (calcium oxalate (mostly) + calcium phosphate). The spectrum IR of the kidney stone (c) defines that is the type IV a (Carbapatite) + type IIIa (anhydrous uric acid), therefore this kidney stone produces a molecular composition of calcium phosphate (mostly) + uric acid (Fig. 1).

Characterization of saponosides

According to the moss test, we note the excessive presence of saponosides in the plant *Paronychia argentea* by the presence of mosses in the 10 tubes with heights between 0.5 and 2.0 cm, this test defines a foam index Im = 200. But concerning the plant *Paronychia capitata*; We know that the *P. argentea* defines an antilithiasic we notice a weak presence of saponosides noticed by the height of mosses in the 10 tubes which is between 0.2 and 0.9 (weak) with a foam index Im< 100. Therefore, the plant *Paronychia argentea* contains an important rate in saponosides compared to the plant *Paronychia capitat*a which defines an antilithiasic effect by their property of formation of the foaming

solutions with the crystalline component of the renal calculi dissolves in water in accordance with the work of Djemoui A., 2003[28].

Results of *in vitro* dissolution tests

Results on the variation in the weight of kidney stones

The average weights of the kidney stones (g) tested for dissolution, the fragments of the kidney stones (a) and (b) in *Paronychia argentea L.* extract; and the fragments of the kidney stones (c) and (d) in *Paronychia capitataL.* extract are summarized in Table 4. The weight variations of the fragments of the kidney stones put in the aqueous extracts of the two plants *Paronychia argenteaL.* (kidney stones (a) and (b)), and *Paronychia capitata L.* (kidney stones (c) and (d)) are represented in Fig. 2. The dissolution kinetics seems to be different depending on the extract of the plant tested.

Results relating to the variation in the dissolution rate in (%) of kidney stones

A change in the weight of the kidney stones appears by a significant decrease from 1.802 ± 0.01 g to 0.583± 0.01 g during the 8 weeks of the experiment for the kidney stone fragments (a). This significant decrease is also accompanied by a decrease in size on average, ranging from 1.20 x 0.46 cm to 0.90 x 0.40 cm. For the kidney stone fragments (b) and the kidney stone (c), there was also a weight reduction ranging from 0.33 ± 0.02 g to 0.12 ± 0.01 g during the first two weeks of the experiment, followed by weight stabilization during the following 6 weeks. On the other hand, for kidney stone fragments (d), a very small decrease in weight was reported during the 8 weeks of the experiment (Fig. 3).

Results related to the change in percent weight loss in kidney stones

The average loss in kidney stone mass (a) in *Paronychia argentea L.* extract is the most significant during the 8 weeks of the experiment, it is about 1.22 g ± 0.01 g (32.3 ± 0.003%). Thus, for the kidney stone (b), there was a loss of mass of 0.33 ± 0.02 g (84.4 ± 0.004%) during the first week of the experiment, whereas for the other kidney stone fragments (b) and (d), the loss of mass was small throughout the experiment (Fig. 4). These results concerning mass loss show a strong correlation with dissolution rates. Indeed, for the kidney stone fragments (a), the highest dissolution rate recorded is 67.7 ± 0.006%. The kidney stone fragments (c) have a dissolution rate of 20.3 ± 0.007%, but the kidney stone fragments (b) and (d) have rates of less than 4 ± 0.003% during the 8 weeks of the experiment (Fig. 3).

Results related to pH variation

The initial pH of dissolving herbal teas given in Table 5. The pH evaluation of the dissolution solutions has been illustrated in Fig. 5, the values for the control of *Paronychia argentea L.* extract range from 7.93 to 6.82 with an average of 7.02 ± 0.01, and for the *Paronychia capitataL.* extract, and they range from 6.71 to 5.91 with an average of 5.91 ± 0.02. A solution of the kidney stone fragments (a), the pH increases with respect to the control solution with 8.35 to 8.40 with an average of 8.40 ± 0.01, these values are closer to those of the control solution of the dissolving extract of the kidney stone fragments (c). Concerning the kidney stone fragments in the aqueous extract of *Paronychia capitataL.*, an increase in pH is observed with respect to the control extract.

Calcium variation results

The variation in calcium levels has been illustrated in Fig. 6.

The variation in calcium levels in all dissolving herbal teas during the 8 weeks was observed, but the most significant increase was observed in the herbal tea containing kidney stone (b) in *Paronychia argentea L.* extract with an average of 1.25 ± 0.02 mg/l.

Magnesium variation results

The variation of the magnesium level during the 8 weeks of experience in the extracts of the two plants is illustrated in Fig 7. Magnesium levels were increased in dissolving herbal teas containing kidney stone fragments (b) and (d) compared to the control herbal tea during the 4 weeks of the experiment with an average increase of 2.12 ± 0.01 mg/l and 1.75 ± 0.03 mg/l respectively, and then stabilized during the following weeks. For the kidney stone fragments (a)

no significant change was recorded compared to the control tea of the *Paronychia argentea L.* plant, as well as the kidney stones (c) compared to the control tea of the *Paronychia capitata L.* plant.

Results for phosphorus variation

The figure 8 shows the variation in phosphorus levels in the dissolution extracts of the two plants during the 8 weeks of the experiment. There was no significant increase over the 8 weeks for all herbal tea dissolving tests of the four kidney stones.

Result of the theoretical evaluation of the dissolution the kidney stone (anhydrous uric acid type)

This effect the dissolution could be linked to the formation of "anhydrous uric acid-oleanan saponoside" complex (Fig. 9) whose stability would be ensured by hydrogen bonds and hydrophilic bonds between the functional groups of the active ingredients and the ketone or amine groups of the uric acid molecule. The complex formed would be much more soluble in water than in anhydrous uric acid in crystallization, thus leading to the dissolution of stones while maintaining in solution sometimes high quantities of dissolved uric acid

DISCUSSIONS

According to clinical and morphological data on kidney stones used in test in vitro of dissolution, we note these kidney stones become from female patients, depending on the Epidemiological Study you say that the incidence of urolithiasis in men is higher compared to women in the general population while in the population affected by the metabolic syndrome, women is higher compared to men in Western of Algeria[27]. Also because of the course time researching kidney stones in our study area: Oran- Western of Algeria, we have found these kidney stones are received from female patients

The results obtained revealed a difference between the effect of the two extracts on kidney stones, reflected by the loss of mass, the dissolution rate and the pH of the medium. *Paronychia argentea L.* has a much greater effect of dissolution on renal calculi (a) than on renal calculi (b). On the other hand, *Paronychia capitata L.* has a less significant dissolving effect on the kidney stone (c), and has no effect on the renal calculi (d). *Paronychia argentea L.* therefore has a better dissolving effect on the renal calculi(a) composed of anhydrous uric acid compared to the renal calculi(b) composed mainly of calcium oxalate. On the other hand, *Paronychia capitata L.* has a weak dissolving effect on the renal calculi (c) based on ammonium phosphate.

A significant dissolution effect of 40% on kidney stones of anhydrous uric acid type compared to kidney stones of calcium oxalate type was recorded. However, the aqueous extract of *Paronychia capitata L.* does not show a significant effect on these kidney stones. This is undoubtedly due to the presence of high saponoside levels in *Paronychia argentea L.* compared to *Paronychia capitata L.* which contains lower saponoside content than that found in *Paronychia argentea L.* extract, which is in perfect agreement with the results of some studies on the dissolution of kidney stones by extracts of some plants[28][29].

An increase in pH values was observed in aqueous extracts of *Paronychia capitata L.* compared to the control extract explained by the release of alkalizing ions such as phosphate ion from the dissolution of ammonium phosphate kidney stones (c) and urate from the anhydrous uric acid renal calculi (a), for calcium oxalate kidney stones; the pH values of the extracts remain unchanged, so the effects of the extracts of these two plants on this type of kidney stone do not match the pH of the dissolution medium but the chemical composition of the extract of each plant[30][31]. The results on the variation in calcium levels can be explained by the ability of the *Paronychia argentea L.* plant to release calcium ions attached to the periphery of calcium oxalate-type kidney stones. On the other hand, *Paronychia capitata L.* does not have this effect. This may be due to the hardness of calcium oxalate type kidney stones. The average increase in magnesium levels is justified by the release of magnesium ions that are contained in the composition of

kidney stones (b) and (d). For kidney stones (a) and (c), no significant change compared to the control tea, so both kidney stones (a) and (c) do not contain magnesium in their compositions [32]. The aqueous extract of *Paronychia argentea L.* is richer in magnesium than the extract of *Paronychia capitata L.* Therefore,*Paronychia argentea L.* has a higher capacity to dissolve magnesium-based kidney stones compared to calcium- or phosphorus-based kidney stones. Organic type stones (such as uric acid or protein-based kidney stones) are more sensitive to aqueous extracts of *Paronychia argentea L.* than mineral type kidney stones (such as calcium oxalate and calcium phosphate) because of their fragility, whereas *Paronychia capitata L.* extracts have a less significant dissolving effect on calcium phosphate and/or magnesium type kidney stones (struvite) and no effect on mineral type kidney stones (calcium oxalate).

Examination of all the chemical constituents present in the two plants used suggests that a mechanism of action of the *Paronychia argentea L.* plant on the dissolution of kidney stones is independent of the pH of the dissolution medium. This effect could be related to the formation of an anhydrous saponoside-uric acid complex (Fig. 9) whose stability would be ensured by hydrogen bonds between the functional groups of the active ingredients and the carboxylic or amine functions of the uric acid molecule. This complex formed would be much more soluble than crystallized anhydrous uric acid [30][33].

Changes in the dimensional and mass characteristics of anhydrous uric acid and oxalate or calcium phosphate kidney stones after treatment with aqueous extracts (*Paronychia argentea L.* and *Paronychia capitata L.*) suggest that these extracts have a definite effect on these crystals, but the evaluation of the results by loss of mass of the stones compared to a reference solution did not reveal a fully significant solvation effect over the entire experimental period, these results are probably due to insufficient contact time and the hardness of the kidney stones

CONCLUSION

Therefore, despite the use of these plants in traditional medicine for the prevention of lithiasis, at this time and within the limits of our experience we cannot recommend these plants for the curative and / or prophylactic treatment of kidney stones. Additional long-term in vitro and in vivo tests are required to demonstrate some of the curative and preventive effects of the extracts tested on urinary stones. Critical clinical trials are required in further research and investigations to validate the efficacy and safety of these plants in patients with kidney stones. However, these plants probably have an interest in preventing recurrences for lithiasis patient with no sign of tolerance by human ingestion.

DECLARATION OF INTERESTS

The authors declare that they have no conflicts of interest in relation to this article.

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REFERENCES

1. Jungers P, Daudon M, Le Duc A P M,A. Lithiase urinaire. Medecine-Sciences, Flammarion. Paris: 1989.
2. Daudon M, Jungers P, Traxer O. Lithiase urinaire. Paris: Médecine sciences publications-[Lavoisier; 2012.
3. Tabuti JR, Lye KA, Dhillion SS. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. J Ethnopharmacol 2003;88(1):19–44.
4. Battandier, Trabut JA L. Flora and catalog of plants from Algeria: or systematic listing of all plants reported to date as spontaneous in Algeria with description of the species found in the Algeria region. Monocots. Adolphe Jordan. Jordan: 1884.
5. Harrache D, Mesri Z, Addou A, Semmoud A, Lacour B. Analyse des calculs urinaires de l'adulte dans l'Ouest Algérien par spectroscopie infrarouge à transformée de Fourier. Eurobiologiste Paris 1997;31(228):11–6.
6. Daudon, Amiel M J. Epidemiology of urolithiasis. Prog En Urol 1999;9(4):5–16.
7. Ghourri M, Zidane L, Douira A. Catalogue des plantes médicinales utilisées dans le traitement de la lithiase rénale dans la province de Tan-Tan (Maroc saharien). Int J Biol Chem Sci 2013;7(4):1688–700.
8. Rossi, Coulange D C. In situ dissolution of urinary calculi. Technical Edition E.M.C, Néphrologie-Urologie. Paris: 1992.
9. Quyou, A. Development of a database on medicinal plants. Example of practical use of this base. Doctoral thesis. 2003;
10. Lucienne Delille D. Les plantes médicinales d'Algérie. Berti Edition. France; 2007.
11. Daudon M. Why and how to analyze urinary calculus? Prog Urol 2007;17(4):2–6.
12. Daudon M. Comment analyser un calcul et comment interpréter le résultat. Eurobiologiste Paris 1993;27(203):35–46.
13. Najjar M.F., M'hennit M.F., Rammah M.A, Oueslati A., Ben Amor, Zouaghi H. MF M F, M A, A,H. Analysis of Lithiasis by IR -Infrared spectrophotometry in the analysis of urinary stones. 1988;
14. SOUMIA B, NAIMA B, ZINA A. IN VITRO ANTIOXIDANT ACTIVITY, IN VIVO ANTIPYRETIC AND ANTI-INFLAMMATORY ACTIVITIES OF PARONYCHIA CAPITATA L. In Vitro 2017;10(3).
15. Bouanani S, Henchiri C, Migianu-Griffoni E, Aouf N, Lecouvey M. Pharmacological and toxicological effects of Paronychia argentea in experimental calcium oxalate nephrolithiasis in rats. J Ethnopharmacol 2010;129(1):38–45.
16. Meiouet F, El Kabbaj S, Daudon M. In vitro study of the litholytic effects of herbal extracts on cystine urinary calculi. Progres En Urol J Assoc Francaise Urol Soc Francaise Urol 2010;21(1):40–7.
17. Badiaga M. Etude ethnobotanique, phytochimique et activités biologiques de Nauclea latifolia Smith, une plante médicinale africaine récoltée au Mali. 2011;
18. Hannache B, Bazin D, Boutefnouchet A, Daudon M. Effet des extraits de plantes médicinales sur la dissolution des calculs rénaux de cystine in vitro: étude à l'échelle mésoscopique. Prog En Urol 2012;22(10):577–82.
19. El Habbani R, Chaqroune A, Houssaini TS, Arrayhani M, Lahrichi A. Contribution to the study of the mass reduction of stones by some medicinal plants. Int J Innov Res Sci Eng Technol 2015;4(04):1867–75.
20. Pareta SK, Patra KC, Mazumder PM, Sasmal D. Prophylactic role of Boerhaavia diffusa in ethylene glycol induced calcium oxalate urolithiasis. Afr J Urol 2011;17(2).
21. Sarkar BR, Chauhan UPS. A new method for determining micro quantities of calcium in biological materials. Anal Biochem 1967;20(1):155–66.
22. Mann CK, Yoe JH. Spectrophotometric determination of magnesium with sodium 1-azo-2-hydroxy-3-(2, 4-dimethylcarboxanilido)-naphthalene-1-(2-hydroxybenzene-5-sulfonate). Anal Chem 1956;28(2):202–5.
23. Gamst O, Try K. Determination of serum-phosphate without deproteinization by ultraviolet spectrophotometry of the phosphomolybdic acid complex. Scand J Clin Lab Invest 1980;40(5):483–6.
24. Di Ventura B, Lemerle C, Michalodimitrakis K, Serrano L. From in vivo to in silico biology and back. Nature 2006;443(7111):527–33.
25. Naseem Aslam C, Allah Bux G, Ali Muhammad S, Lubna N. Analysis of kidney stones by FTIR spectroscopy. 2007;

26. Sekkoum K, Cheriti A, Taleb S, Belboukhari N. FTIR spectroscopic study of human urinary stones from El Bayadh district (Algeria). Arab J Chem 2016;9(3):330–4.
27. Meiouet F, El Kabbaj S, Daudon M. Étude in vitro de l'activité litholytique de quatre plantes médicinales vis-à-vis des calculs urinaires de cystine. Prog En Urol 2011;21(1):40–7.
28. Djemoui A. The inhibitory effect of the extract of some Saharan plants on urolithiasis type Oxalo-Calcic. 2003;
29. Sait S, Hamri-Zeghichi S, Boulekbache-Makhlouf L, Madani K, Rigou P, Brighenti V, et al. HPLC-UV/DAD and ESI-MSn analysis of flavonoids and antioxidant activity of an Algerian medicinal plant: Paronychia argentea Lam. J Pharm Biomed Anal 2015;111:231–40.
30. Patel PK, Patel MA, Vyas BA, Shah DR, Gandhi TR. Antiurolithiatic activity of saponin rich fraction from the fruits of Solanum xanthocarpum Schrad. &Wendl.(Solanaceae) against ethylene glycol induced urolithiasis in rats. J Ethnopharmacol 2012;144(1):160–70.
31. Gupta S, Kanwar SS. Phyto-molecules for kidney stones treatment and management. Biochem Anal Biochem 2018;7(362):2161–1009.
32. Daudon M, Traxer O, Lechevallier E, Saussine C. La lithogenèse. Prog En Urol 2008;18(12):815–27.
33. Ahmed S, Hasan MM, Mahmood ZA. Antiurolithiatic plants: Multidimensional pharmacology. J Pharmacogn Phytochem 2016;5(2):4.

Table 1: Clinical and morphological data on kidney stones used

Parameters	Kidney stones (a)	Kidney stones (b)	Kidney stones (c)	Kidney stones (d)
Donor	male/Female	male/Female	male/Female	male/Female
Age	32-45 years	55-65 years	55-65 years	55-65 years
Dimensions (mm)	120 x 46 x 35	120 x 81 x 65	120 x 50 x 42	65 x 30 x 18
Color	Brownish (brown-black)	brown-black	Yellowish (yellow-black)	Dark brown
Dry weight (g)	1.802 ± 0.5	3.445 ± 0.5	2.113 ± 0.5	1.174 ± 0.5
External form	Coralliform has buds	Irregular smooth with budding	Ovoid with pointed branches	Mammoth Ovoid
Anatomical localization	left kidney	pyelocaliciel cavity (Left kidney)	left kidney	left kidney

Table 2: Morpho-Constitutional Analysis of the Kidney Stones Used in the *in vitro* Dissolution Tests

Property	Kidney stones (a)	Kidney stones (b)	Kidney stones (c)	Kidney stones(d)
Aspect of peripheral	Heteromorphic rounded, very little dented Cylindrical with buds	Irregular oval flattened, Homogeneous smooth,	Irregular oval-flattened	ovoid budded Homogeneous nipple
Aspect of the section	Unorganized crystalline loose (amorphous)	Concentric compact with radial crystallization	Prismatic crystals more or less sounded unorganized and loose	Concentric compact with radial crystallization that diffuses towards a gap in the center
Hardness	Brittle-breaking	Hard-not brittle	Brittle-breaking	Hard-not brittle
Calcination	charcoal flame-free burning -black residues > 80%.	-don't charcoal -not hot -abundant greyish residues	- does not charcoal -not hot - an abundant whitish residue	-does not charcoal -not hot -an abundant greyish residue

Table 3: Chemical Analysis Results of Kidney Stones Used *in vitro* Dissolution Tests

Constituents	Kidney stones (a)	Kidney stones (b)	Kidney stones (c)	Kidney stones (d)
Carbonates	-	++	++	+++
Calcium phosphate	+	+++	+++	+++
Calcium oxalate	-	+++	+++	++++
Magnesium	-	+	-	++
Uric acid / urates	+++	++	+	-
Xanthine	-	-	+	+

Table 4: Average weight of kidney stones (g) tested for dissolution

Aqueous extract -*Paronychia argentea L.*-		Aqueous extract-*Paronychia capitata L.*-	
Kidney stones fragments (a)	Kidney stones fragments (b)	Kidney stones fragments (c)	Kidney stones fragments (d)
1.802± 0.5	2.113 ± 0.5	3.445 ±0.5	1.174 ± 0.5

Table 5: Initial pH values of dissolving herbal teas

Infusion extract of *Paronychia argentea L.*			Infusion extract of *Paronychia capitata L.*		
Reference solution	Kidney stones (a)	Kidney stones (b)	Reference solution	Kidney stones (c)	Kidney stones (d)
7,93 ± 0,03	8,35 ± 0,04	7,64 ± 0,03	6,71 ± 0,04	8,02 ± 0,02	7,98 ± 0,04

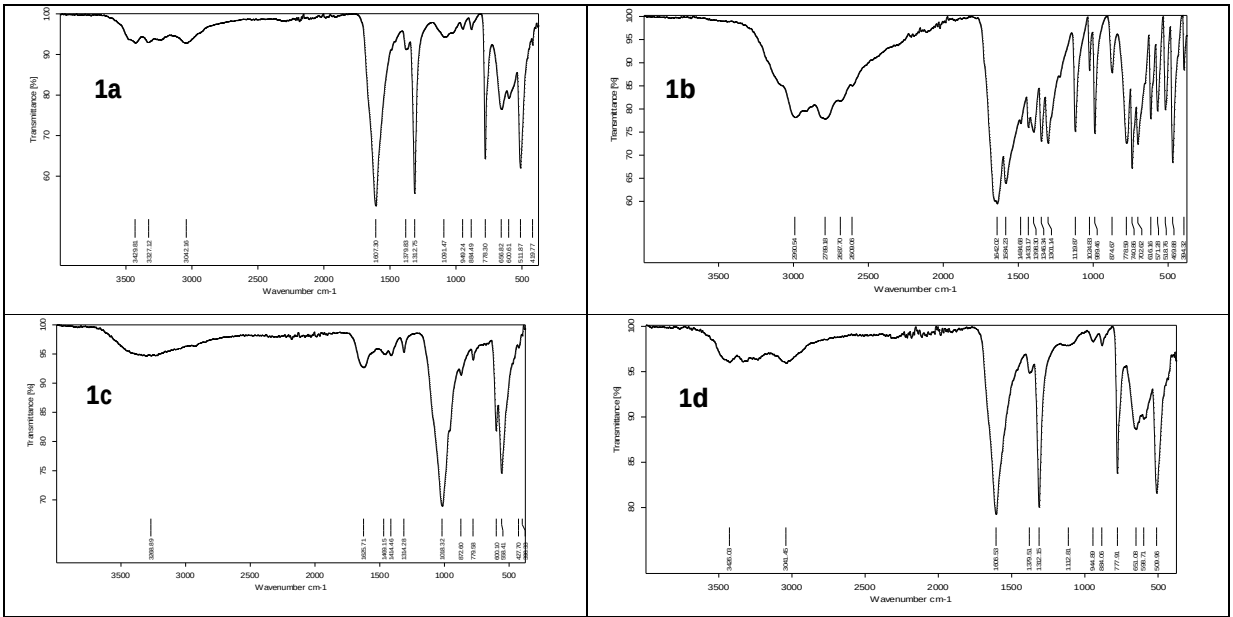

Figure 1: The IR spectra of these urinary stones [(1a) : the kidney stone (a) spectrum, (1b) : the kidney stone (b) spectrum, (1c) : the kidney stone (c) spectrum, (1d) : the kidney stone (d) spectrum]

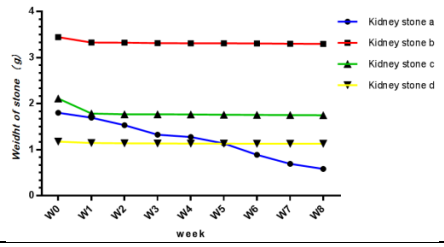

Figure 2: The variation in weight (g) of urinary stones

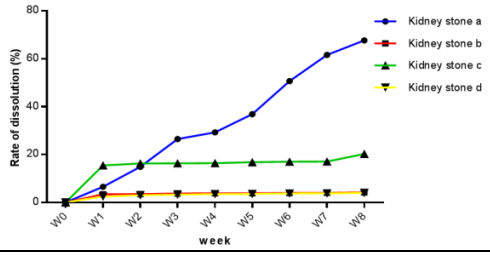

Figure 3: Variation in dissolution rate (%) of kidney stones

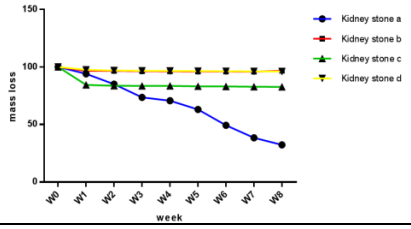

Figure 4: Evaluation of the weight loss of these kidney stones

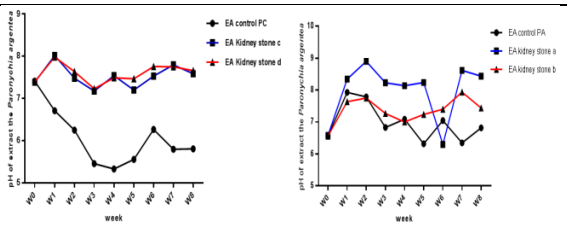

Figure 5: Evolution the pH of the extracts of these plants tested on kidney stones during 8 weeks

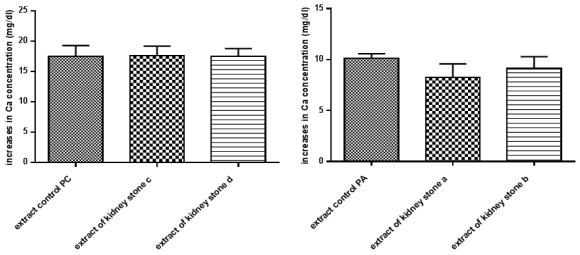

Figure 6: Evaluation of calcium levels in the dissolution extracts during the 8 weeks

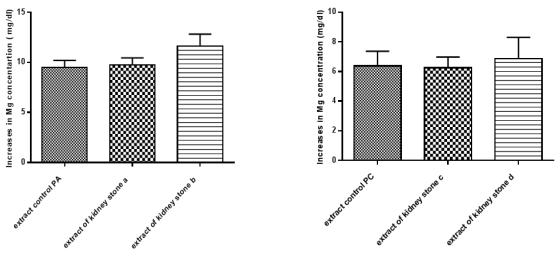

Figure 7: Evaluation of magnesium levels in dissolution extracts during the 8 weeks

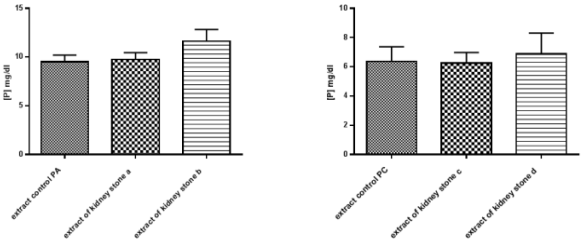

Figure 8: Evaluation of phosphorus levels in dissolution extracts during the 8 weeks

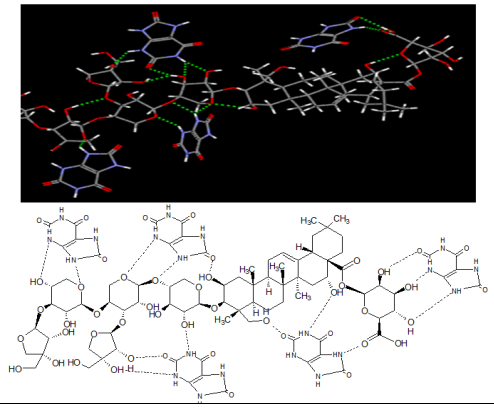

Figure 9: Theoretical evaluation of dissolution phenomenon by formation the complexes anhydrous uric acid-saponoside oleanane complex

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RESEARCH ARTICLE

HRM Practices of Employees in Micro, Small and Medium Enterprises

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ABSTRACT

Micro Small and Medium enterprises (MSME) Sector has emerged as a highly vibrant and dynamic sector of the Indian economy. These sector not only play crucial role in providing large employment opportunities at comparatively lower capital cost than large industries but also help in industrialization of rural areas. The MSMEs are classified as micro, small and medium enterprises based on their investment in business. The unavailability of adequate and timely credit facility, high cost of credit, lack of modern technology insufficient training and skill development are the main problems of MSME.

Keywords: MSME, low capital cost, large employment in MSME.

INTRODUCTION

The employment is more possible through the development of MSME. The MSME will be able to satisfy the needs of the customers upto a great extent after considering their expectations primarily. Migration of rural youths can stopped by providing them chance to work at their place. The mutual change of technology among the different types of MSME s financial & technical assistance training & skills formation will assist in the development of MSME. The MSME sector has shown admirable innovativeness and adaptability to survive the recent economic turn and recession with its agility and dynamism to have lot of opportunities.

Objectives

- To analyse the present conditions of employee in MSME towards Human resource management.
- To identify the measures for the improvement of HRM practices in MSMEs.

Statement of the Problem

The organizations ultimate performance based on the HRM practices in MSME sector. It enriches the efficiency, innovativeness, employment opportunities and thus it increases the production performance of MSME. The employees satisfaction or dissatisfaction affects the organizations performance. So every organization is giving high priority to keep their employees with satisfaction by providing several facilities which improves satisfaction and reduces dissatisfaction.

Limitations of the Problem

The main limitations of the study are:

- It is limited to the field of HRM practice alone.
- The sample size is limited number only.
- The accuracy level of the data provided by the respondent is not known.
- This study has been conducted at coimbatore.

RESEARCH METHODOLOGY

Both primary and secondary data were used for the present study. Convenient random sampling method has been used for this study, secondary data have been collected from websites, records and journals. HRM professionals from Coimbatore city are involved in this study. The data required for the study have been collected through the structured questionnaire.

Sample Size: 50 employees were deliberated for the study purpose from gear manufacturing industries.

Tools for analysis: Chi square analysis and Anova.

Analysis and Discussion

Chi-Square Test

Comparison of between training and development and job satisfaction.

Null Hypothesis

H0: There is no significant difference between training and development and job satisfaction.

Alternative Hypothesis

H1: There is significant difference between training and development and job satisfaction.

Interpretation

Hence the value is less than 0.05, we accept null hypothesis and reject alternate hypothesis. So there is no significant difference between training and development and job satisfaction.

ANOVA

Measure the degree of relationship between Monthly income and Employee performance.

Null Hypothesis Ho

There is no significant relationship between monthly income and employee performance

Alternative Hypothesis H_1

There is a significant relationship between monthly income and employee performance. The mean difference is significant at the 0.05 level.

Dunnet t t-tests treat one group as a control, and compare all other groups against it.

Means for groups in homogeneous subsets are displayed. Uses Harmonic Mean Sample Size = 36.367.

Interpretation

From the above analysis, we find that calculated value of the F-value 13.197 is a positive value, so H1 accept. Since the P value 0.000 is less than < 0.05 regarding there is a significant relationship between monthly income and employee performance. The results are significant at 5 % level.

FINDINGS

- Chi square analysis results that there is no significant difference between training and development and job satisfaction.
- Anova analysis results that the calculated value of the F-value 13.197 is a positive value, so H1 accept. Since the P value 0.000 is less than < 0.05 regarding there is a significant relationship between monthly income and employee performance.

SUGGESTIONS

The employees have to able to control over about the time management. Drop few of risk taking work that save the time and energy. Getting relaxation when the works go long way. Each and every organization under MSME sector can improve the working conditions, environment and other policies based on my survey. I can get an opportunity to provide suggestions, they can implement my suggestions to overcome many obstacles face by the organization. More overit must directed at understanding individual differences so that employee initiatives to improve their work performance.

CONCLUSION

Analysing the HRM practices are most likely to be successfully mainstreamed which have a clear understanding of their work motivation and which differences the important of employees performance. No matter what the preferred way, it is hoped this research paper will form a stepping stone in the process and provide a basis for HRM practices in MSME sector Coimbatore.

REFERENCES

1. Kotrlik, J., & Higgins, C. (2001). Organizational research: Determining appropriate sample size in survey research appropriate sample size in survey research. Information technology, learning, and performance journal, 19(1), 43
2. Lambrou, P., Kontodimopoulos, N., &Niakas, D. (2010). Motivation and job satisfaction among medical and nursing staff in a Cyprus public general hospital. Human resources for health, 8(1), 26.
3. Mira,M., Choong,Y., &Thim,C.(2019). The effect employee performance. Management Science Letters,9(6),771-786.
4. Nataraja, S., &Alamri, M. S. (2016). Strategic human resource management in Saudi Arabia's service sector. Journal of Competitiveness Studies, 24(1-2), 91-102

Table 1. Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	91.829[a]	9	.000
Likelihood Ratio	102.589	9	.000
Linear-by-Linear Association	7.715	1	.005
N of Valid Cases	148		

Table 2. ANOVA

Employee performance					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	13.581	2	6.791	13.197	.000
Within Groups	74.608	145	.515		
Total	88.189	147			

Employee performance					
	Employees relationship	N	Subset for alpha = 0.05		
			1	2	3
Tukey B[a]	Strongly Agree	36	1.81		
	Agree	89		2.26	
	Neutral	23			2.78
Duncan[a]	Strongly Agree	36	1.81		
	Agree	89		2.26	
	Neutral	23			2.78
	Sig.		1.000	1.000	1.000
Waller-Duncan[a]	Strongly Agree	36	1.81		
	Agree	89		2.26	
	Neutral	23			2.78

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RESEARCH ARTICLE

Assessment of Rare and Endangered Pteridophytes of Kumaun Himalaya

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ABSTRACT

Like the other parts of the Himalaya, the flora of the region is also under great biotic pressure due to selective removal as well as habitat clearance for cultivation. The habitat fragmentation particularly in areas where most of the plants grow has probably caused their being rare and endangered. The depletion and disappearance of these plants have largely been caused by road construction, dam building and establishment of industries on a large scale in recent past. The bulk of collections of medicinal and useful plants has also played an important role in the depletion of plants. A large area once occupied by dense forests have been converted into grazing lands. Because of destruction of natural habitats on exponential scale, indiscriminate deforestation and over grazing leading to disturbed ecological balance and the floods, soil erosion and landslides also convert considerable areas into stony deserts. Thus the destruction of natural habitat by any one of the above mentioned causes results in entirely different habitat invaded by other groups of plants and making the conditions unfavourable and unsuitable for their exixtence

Keywords: Himalaya, industries, forests, natural, flora

INTRODUCTION

The Garhwal and Kumaun Himalayan ranges lie in the North Indian state of Uttarakhand, newly formed in 2000 from the northern part of the state of Uttar Pradesh. They form the eastern end of the west Himalaya (here defined practically as the Himalaya west of the western Nepalese border) and abound in a rich and varied flora and fauna, constituting the most species-rich part of the whole of the west Himalaya. This segment of the Himalaya is a transitional zone and floristic meeting-ground between the drier and more temperate west Himalaya and the wetter and more subtropical east Himalaya. Here, many west-Himalayan floral elements (including European-type elements and a few west-Himalayan endemics, or near endemics, of the Sino-Himalayan type) and east-Himalayan floral elements (including both Sino-Himalayan and S.E. Asian-type elements) show their eastward and westward limits of distribution respectively.

The region thus exhibits a greater admixture of both west and east Himalayan floral elements than any other part of the Indo-Himalaya. But the overall affinity of the flora of this region with that of the highly species-rich east Himalaya is much stronger than for any other part of the west Himalaya, thus the total number of pteridophytes in the area is higher than any other part of the West Himalaya. In spite of such a varied and rich flora including a high number of pteridophytes, no accurate modern attempt has been made to describe the existing pteridophyte species in a comprehensive manner.

The total number of pteridophyte species pres-ent in India is c. 1100 and of these 337 tax a are considered to be threatened or endangered (nearly one third of the total). It should be realised that IUCN listing (IUCN, 2010) is or ganised by countries and the global rarity and endangerment of species is therefore often some-what masked in an area where the floras are inti-mately related. This particularly applies to the two major groups of Sino-Himalayan and S. E. Asian/Malesian elements present in India which extend across the eastern borders into China, Myanmar etc. From the entire Kumaun Himalaya, Pangtey and Punetha (1987) attempted for the first time after Duthie (1906) to enumerate all the pteridophytes then known to them based on their collections coupled with previous records. It was followed by the compilation of a list of ferns Kumaun Himalaya by Pande (1990) and Pande and Pande (1991). Recently, Pande and Pande (2002) again enumerated the same list of pteridophytes of Kumaun Himalaya. Further Pande and Pande (2003) have published an *Illustrated Fern flora of Kumaun Himalaya* and described 341 species belonging to 93 genera and 39 families excluding fern-allies. From the entire Uttarakhand, Dixit and Kumar (2002) published *Pteridophytes of Uttaranchal* (*A Checklist*) and have attempted to enumerate the total known species of ferns and fern-allies and listed a total of 105 genera, 444 species, 5 subspecies and 26 varieties spread over 49 families. This enumeration is largely based on the previous publications without making collections and researches of their own in the pteridophytic flora of Uttarakhand and they entered all published species without verifying the voucher specimens. While compiling the pteridophytes of Uttarakhand, they overlooked many genera and species. Thus they recorded many misapplied names, basionyms and even synonyms as independently good species unfortunately.

MATERIALS AND METHODS

Extensive field visits were conducte for the authentic information**.** The present work is based on the detailed and careful collections of pteridophytic flora of the study area between the altitudes, stretching from 800-2,356 m, throughtout the year particularly during rainy season, when almost all pteridophytes show abundant and luxuriant growth and development. During the field explorations, observations on the habit, habitat, size of fronds, nature of rhizomes, scales, branching of fronds, abundance and ecology *etc.* of pteridophytic flora were recorded. The method of collections, pressing, poisoning and mounting was carried following Jain and Rao (1976). All the specimens collected were critically examined and identified mainly with the help of Clarke (1880); Beddome (1883 & 1892); Hope (1899-1904); Khullar *et al*. (1991); Khullar (1994 & 2000); Pande and Pande (2003); Fraser-jenkins (2009); Joshi *et al.* (2009) and several other important published works.

RESULT

Assessment of Rare and Endangered Species

With the result, some plants have become threatened and endangered, while the rare ones are on the verge of extinction. If these conditions continue to operate for some more years, these threatened, endangered and rare plant species appear likely to disappear from this area in the foreseeable future. Some of such rare and endangered pteridophytes of the study are given below. All these plants have been collected only once or twice having a very small population in nature and grow in a very specialised habitats in miserable conditions due habitat fragmentations. (Table 1 and fig 1). Total 23 species were recorded under20 genera and 20 family.

Conservation strategies

The pteridophytes are moisture and shade loving plants and dependent upon the microclimatic conditions of the region for their successful survival in that region. Any kind of disturbance in these microclimatic conditions can hinder the growth and evolutionary processes occurring naturally in these plants thereby, leading to decline in their populations. Thus, factors like climate change, increasing urbanization, industrialization, encroachment of forest lands, unplanned developmental activities, over exploitation of natural resources, pose a major threat to the survival of these groups of plants. Due to unplanned felling of trees in the forests the members of epiphytic pteridophytes belonging to the families Polypodiaceae, Davalliaceae, Aspleniaceae, Vittariaceae, have been reduced day-by-day (Dixit, 2000). Large scale collection of ferns from the forests by the visitors and local people for ornamental purpose, medicinal purpose and during excursions also increases the pressure on these plants.

Biodiversity conservation is the need of time and hence, it has become imperative to develop *in situ* and *ex situ* conservation methods for conservation of the diminishing biodiversity. The *in situ* conservation is very beneficial as it allows the evolution of the species to continue within the area of natural occurrence. Hence, the steps for conserving the ferns *in situ* should be focused upon. The *ex situ* conservation includes development of botanical gardens or conservatories, germplasm banks, DNA banks, seed banks and involve the use of techniques such as tissue culture, cryopreservation; incorporation of disease, pest and stress tolerance traits through genetic transformation and ecological restoration of rare plant species and their populations (Kapai *et al,* 2010). The conservation of flowering plants has been achieved to good extent by developing conservatories and botanical gardens which also help in creating awareness among the local people. However, developing a fern conservatory or fern garden is not preferred much and hence, such steps should be considered and implemented for conserving the rare and endangered species. The tissue culture is a very useful technique for the mass multiplication of the plant species in a short time and hence, researches focusing on developing a protocol for *in vitro* regeneration of ferns and fern-allies should be encouraged. Parts of areas rich in abundant pteridophyte diversity can be declared as pteridophyte biosphere reserves or small gene sanctuaries can be established to save the epiphytic pteridophytes. The issue is of major concern and the conservation using steps should be taken as early as possible. Some of the measure includes:

1.*In-situ* conservation by establishment of fern house in botanical garden
2.Human interference in the forest area should be prevented
3.Active participation of local people by setting up of floricultural center for the fern too along with other plant species
4.Identification of hot-spots and rare ferns and their proper management should be implemented
5.Construction of green houses to protect the rare species
6.Promotion of establishment of forest conservatories to protect this valuable bio resource in natural

REFERENCES

1. Beddome, R. H. (1883). *A Handbook to the Ferns of British India, Ceylon and Malay Peninsula*. Calcutta.
2. Beddome, R. H. (1892). *A Handbook to the Ferns of British India, Ceylon and Malay Peninsula with Supplement*. Calcutta.
3. Clarke, C. B. (1880). A review of ferns of northern India. *Trans. Linn. Soc. Lond. II. (Bot.)* 1: 425-611.
4. Dhir, K. K (1980). Ferns of North Western Himalayas. *Bibliotheca Pteridologia* 1: 1-158.
5. Dixit, R.D. and R., Kumar (2002). *Pteridophytes of Uttarakhand (A Check List).* Dehradun.
6. Duthie, J. F. (1906). Catalogue of the Plants of Kumaon and of the Adjacent Portions of Garhwal and Tibet, based on the collections made by Strachey and Winterbottom during the years 1846 to 1849 and on the catalogue originally prepared in 1852 by Sir Richard Strachey. London.

7. Fraser-Jenkins, C.R.(2009).Taxonomic Revision of Three Hundred Indian Subcontinental Pteridophtes with a Revised Census-List. Dehra Dun.
8. Hope, C. W. (1899-1904). The ferns of Nort-Western India. *J. Bombay nat. Hist. Soc*. 12: 315-325, 527-538, 62-633 (1899); 13: 25-36, 236-251 (1900); 443-461, 657-671 (1901); 14: 119-127, 252-266, 458-480 (1902); 720-749 (1903); 15: 67-111, 415-429 (1904).
9. Jain, S.K. and R.R.Rao (1976). *A Handbook of Field and Herbarium Methods*. New Delhi.
10. Jain, S.K. and R.R.Rao (1976). *A Handbook of Field and Herbarium Methods*. New Delhi.
11. Joshi, P., H. C. Pande and P.C. Pande (2009). *Ferns of Cenral Himalayas-I* (*Chamoli and Rudrarayag*). Dehra Dun.
12. Kaur, S. and N. Punetha (1984). A new variety of *Dicranopteris linearis* (Burm. f.)
13. Khullar, S. P. (1994). *An Illustrated Fern Flora of West Himalaya*. Vol. I. (Botrychiaceae to Aspleniaceae). Dehradun.
14. Khullar, S. P. (2000). *An Illustrated Fern Flora of West Himalaya*. Vol. II (Onocleaceae to Salviniaceae). Dehradun.
15. Khullar, S. P., Anurita Sharma and S. S. Samant (1991). A new species of the genus *Elaphoglossum* from the Kumaun Himalaya. *Indian Fern J*. 8: 1-4.
16. Pande, H.C. and P.C.Pande (2003). *An Illustrated Fern Flora of the Kumaon Himalaya*. Vo. I & II. Dehradun.
17. Pande, P.C. and H.C. Pande (2002). An updated catalogue of ferns and fern allies of Garhwal Himalaya. *Indian J. Forestry* 25:486-498.
18. Pangtey, Y. P. S. and N. Punetha (1987). Pteridophytic flora of Kumaun Himalaya : An up dated list.In : *Western Himalaya (Environment, Problem & Development), ed*. Y. P. S. Pangtey & S. C. Joshi, Naini Tal. 1: 389-412.
19. Punetha, N. and S. Kaur (1987). Pteridophytic flora of Pithoragarh district (North-Western Himalaya). *J. Econ. Tax. Bot*. 9: 269-286.
20. Rao, T. A. (1959). Report on a botanical tour to Milam glacier. *Bull. Bot. Surv. India* 1: 97.

Table 1: Family and species list of rare and endangered pteridiphytes

Species	Family
Angiopteris evecta (Forst.) Hoffm	Marattiaceae
Arachniodes amabilis (Forst. *fil*.) Tindale	Dryopteridaceae
Araiostegia membranulosa (Wall. *ex* Hook.) Holtt.	Davalliaceae
Arthromeris lehmanii (Mett.) Ching	polypodeaceae
Asplenium nidus L	Aspleniaceae
Athyrium distans (D.Don) Moore	Athyriaceae
Blechnum orientale L.	Blechnaceae
Cyathea spinulosa Wall. *ex* Hook	Cyatheaceae
Dennstaedtia spectabile (Wall. *ex* Mett.) Ching	Dennstaedtiaceae
Dennstaedtia subsinuatum (Wall. *ex* Hook. & Grev.) Tag	Dennstaedtiaceae
Dennstaedtia scabra Wall. *ex* Moore	Dennstaedtiaceae
Diplazium polypodioides Blume	Athyriaceae
Elaphoglossum marginantum (Wall. *ex* Fee) Moore	Dryopteridaceae
Elaphoglossum stelligerum (Wall. *ex* Hook.) Moore *ex* Alston & Bonner	Dryopteridaceae
Lycopodiella cernua (L.) Pichi Serm	Lycopodiaceae
Microlepia khasiana (Hook.) Presl	Dennstaedtiaceae
Osmunda japonica Thunb	Osmundaceae
Peranema cyatheoides D.Don	*Dryopteridaceae*
Plagiogyria scandens Mett	Plagiogyriacea
Psilotum nudum (L.) P.Beauv	Psilotaceae
Pyrrosia costata (Wall. *ex* Presl) Tag. & Iwats	Polypodiaceae
Tectaria polymorpha (Wall. *ex* Hook.) Copel	Tectariaceae
Trichomanes radicans Sw.	Hymenophyllaceae

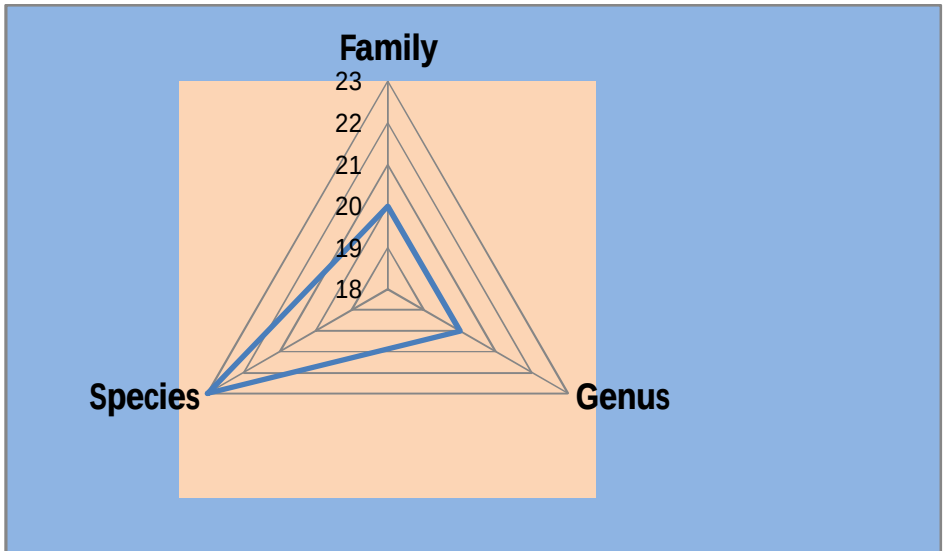

Fig 1: Graphical presentation of species genus and family distribution

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RESEARCH ARTICLE

Adoptability to Salinity in Response to Germination of Brinjal (*Solanum Melongena* L.)

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ABSTRACT

The present study deals with the effect of exogenous addition of sodium chloride ranging from 0-120 mM, on the germination studies and biochemical constituents were made to analyzed 7th and 15th day after treatment. The shoot length, root length, number of seed germination and also the percentage of seed germination increased up to 20 mM NaCl and thereafter reduced drastically. The fresh and dry weight of the seedlings measured on 15th day after salt treatment its also increased upto 20 mM NaCl and beyond this level reduced the morphological parameters.

Keywords: Salinity, Leaf area, Dry weight, Chlorophyll, Germination percentage.

INTRODUCTION

Soil salinity is one of the most important problem of crop production in arid and semi arid zones. In India, an estimated total 7.2 million hectares land is salt affected, out of this 4.5 million hectares in saline but non-alkaline and 2.7 million hectares is alkaline. Salt tolerance of plants is a complex and involves a large number of events. The salt tolerance of plant is a complex phenomenon that involves morphological and developmental changes as well as physiological and biochemical processes. These aspects have been covered periodically in several reviews (Zhang *et al.,* 2010; Kholova *et al.,* 2010). Decreasing the amount of photosynthetic pigment is one of the effect of salinity in plants and has been reported in many crop species including tomato (Juan *et al.,* 2008), *Lablab purpureus* (D'Souza and Devaraj, 2010; Negrao *et al.,* 2016; Chunzhao Zhao and Sergey Sabala, 2020; Costa-Motos *et al.,* 2020). The objective of the present work to find out the salinity stress on germination growth and chlorophyll pigments of *Solanum melongena* L.

MATERIALS AND METHODS

The seeds of *Solanum melongena* L. were obtained from Lalbagh, Botanical Garden, Bangalore, Karnataka. The certified seeds were surface sterilized for 2 minutes using 0.2% mercuric chloride ($HgCl_2$) solution and their thoroughly washed with distilled water for thrice. The sterilized seeds were placed on the petridish, each petridish allow 25 seeds and they were treated with various concentrations of NaCl ranging from 10 to 120 mM. The seeds germinated up to 100 mM, the 120 mM NaCl the seeds could not survive. The control seeds were treated without addition of NaCl (only distilled water). The number of seeds germinated to measure both 7th and 15th day after sodium chloride treatment.

Germination percentage

The initial appearance of radical by visual observation refers to germination which calculated by using the formula. Germination percentage is calculated by total number of seed germinated by total number of seeds sown and the both are multiplied by 100.

$$\% = \frac{\text{Total number of seed germinated}}{\text{Total number of seeds sown}} \times 100$$

The formula used to calculate by the method of Carley and Watson (1968).

Morphological studies

The shoot and root length were measured by scale and expressed in cm plant^{-1}. The fresh weight of the germinated seedlings both shoot and root were separated and weighing them in a electronic balance. The dry weight of the germinated seedling both shoot and root were separated and are dried in 80°C for 48 h in an oven and then weighed in electronic balance. The leaf area was calculated by measuring the length and width and number of leaves and multiplied by a correlation factor (0.66) derived from the method of Yoshida *et al.* (1972). The total chlorophyll content of the leaves was estimated by the method of Arnon (1949).

RESULTS AND DISCUSSION

The observation on the germination of brinjal seeds to measured the shoot length, root length and percentage of germination made at 15th day after salt treatment. The results of this study showed that the seedlings was affected by salinity and the effect was varied depending on salinity level. The seedlings survived upto 100 mM NaCl and the optimum growth was found in 20 mM NaCl. The minimum growth of shoot length (9.4 cm plant^{-1}), root length (2.15 cm plant^{-1}), percentage of germination (12%), leaf area (0.97 cm^2 plant^{-1}), fresh weight (1.25; 0.55 g plant^{-1}) and dry weight (0.40; 0.26 g plant^{-1}) were recorded at 100 mM NaCl and maximum increase of the above parameters are found in 20 mM NaCl and this was shoot length (26.7 cm plant^{-1}), root length (7.25 cm plant^{-1}), percentage of germination (25%), leaf area (4.05 cm^2 plant^{-1}), fresh weight (4.75; 1.90 g plant^{-1}) and dry weight (2.00; 0.90 g plant^{-1}) on 15th day after salt treatment (Table 1).

The similar trend was observed in the chlorophyll content also on 15th day after treatment and this was 0.109 mg g^{-1} fr. wt. at optimum level of 20 mM NaCl and at higher concentration drastically reduced the chlorophyll synthesis. Growth inhibition following exposure of plants to salinity is widely reported in most species (Khan *et al.,* 2009) and can be attributed to consequences of change in osmotic potential around the roots as well as specific ion toxicity. The reduced plant height exposed to saline medium might be due to the continued effect of decreased shoot and root length, leaf number and leaf area (Dulormne *et al.,* 2010). The reduction of the leaf area could have consequences on growth, through less effective radiation interception by the leaves and lower carbon fixation (Parida and Das, 2005). In our study, the brinjal plants showed reduction in surface area of the leaves exposure to salinity. The reduction in dry weight can be attributed to the reduced photosynthetic capacity of leaves under salinity stressed condition (Querghi *et al.,* 2000). The earlier reports in

cucumber (Kaya *et al.,* 2003) and tomato (Agong *et al.,* 2003) supported to our results. It is generally known that our photosynthetic efficiency depends on photosynthetic pigments such as chlorophyll 'a' and 'b' which play an important role in photochemical reactions of photosynthesis.

REFERENCES

1. Agong SG, Kingestsu M, Yoshida Y, Yazawa S, Musuda M. Response of tomato genotypes to induced salt stress. Afrc Crop Sci J 2003; 11: 1-9.
2. Arnon DI. Copper enzymes in intact chloroplasts, polyphenoloxidase in *Beta vulgaris.* Plant Physiol 1949; 24: 1-15.
3. Carley HE, Walton. Effect of various aqueous extracts upon seed germination. Bot. Gaz., 1968; 129: 57-62.
4. Chunzhao Zhao, Sergey Shabala. Mechanism of plant responses and adaptation to soil salinity: Review Article. The Innovation 2020; 1: 100008.
5. Costa-Motos JR, Orturo MF, Bemal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco MJ, Hernandez JA. Plant responses to salt stress: Adaptive mechanism. Agronomy 2020; 7-18: 7010018.
6. D'Souza MR, Devaraj VR. Biochemical responses of hyacinth bean (*Lablab purpureus*) to salinity stress. Acta Physiol Plant 2010; 32: 341-353.
7. Dulormne M, Musean O, Muller F, Toribio A, Ba A. Effect of NaCl on growth, water status, N_2 fixation and ion distribution in *Pterocarpus officinalis* seedlings. Plant Soil 2010; 327: 23-34.
8. Juan M, Rivero RM, Romero L, Ruiz JM. Evaluation of some nutritional and biochemical indicators in selecting salt resistant tomato cultivars. Env. Exp. Bot 2005; 54: 193-201.
9. Kaya C, Higgs D, Ince F, Amador BM, Cakir A, Sakar E. Ameliorative effects of potassium phosphate on salt stressed pepper and cucumber. J Plant Nutr 2003; 26: 807-820.
10. Khan NA, Nazar R, Anjum NA. Growth, photosynthesis and antioxidant metabolism in mustard (*Brassica juncea* L.) cultivars differing in ATP sulfurylase activity under salinity stress. Sci Horti 2009; 122: 455-460.
11. Kholova J, Sairam RK, Meena RC. Osmolytes and metal IMS accumulation, oxidative stress on antioxidant enzymes activity as determinants of salinity stress tolerance in maize genotype. Acta Physiol Plant 2010; 32: 477-486.
12. Negrao S, Schmockel M, Tester M. Evaluating physiological responses of plants to salinity stress. Annals J Bot 2016; 119(1): 1-11.
13. Parida AK, Das B. Salt tolerance and salinity effects on plants. Ecol Env Safety 2005; 60: 324-349.
14. Querghi Z, Cormic G, Brulfert J. Effect of NaCl on photosynthesis of two wheat species (*Triticum durum* and *Triticum aestivum*) differencing in their sensitivity of salt stress. J Plant Physiol 2000; 156: 335-446.
15. Yoshida S, Fordo D, Cock J, Gomez K. Laboratory Manual for Physiological Studies of Rice, 3rd edn. The International Rice Research Institute, Philippines, 1972.
16. Zhang QS, Flowers TJ, Wang SM. Mechanism of sodium uptake by roots and higher plants. Plant Soil 2010; 326: 45-60.

Table 1. Effect of salinity on germination of shoot, root, number of leaves and percentage of germination in *Solanum melongena*

Concentration of NaCl (mM)	Shoot length (cm plant^{-1})		Root length (cm plant^{-1})		No. of leaves plant^{-1}	Percentage of germination (25 seeds/petridish)	
	7th day	15th day	7th day	15th day	2	7th day	15th day
0	6.10	18.8	3.10	5.25	2	6	19
10	7.25	22.5	3.75	6.1	2	9	22
20	10.90	26.7	4.50	7.25	2	12	25
40	9.25	23.2	3.20	6.55	2	10	20
60	8.00	19.00	2.14	4.12	2	8	18
80	6.50	17.65	1.50	3.25	2	7	14
100	4.27	9.4	1.00	2.15	–	5	12
120	–	–	–	1.75		1	3

Table 2. Salinity in response to germination and chlorophyll synthesis of *Solanum melongena* on 15th day after treatment

Concentration (mM)	Leaf area (cm plant^{-1})	Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})		Chlorophyll		Total chlorophyll (mg g^{-1} fr. wt)
	15th day	Shoot	Root	Shoot	Root	Chl. 'a'	Chl. 'b'	
0	2.15	2.90	1.04	1.04	0.70	0.040	0.037	0.077
10	3.55	3.25	1.27	1.27	0.84	0.052	0.046	0.098
20	4.05	4.75	1.90	2.00	0.90	0.067	0.052	0.109
40	3.94	4.00	1.33	1.47	0.65	0.060	0.040	0.100
60	2.65	3.10	0.97	1.14	0.55	0.047	0.031	0.078
80	2.13	2.14	0.80	0.65	0.40	0.039	0.024	0.063
100	0.97	1.25	0.55	0.40	0.26	0.022	0.017	0.039
120	–	–	–	–	–	–	–	–

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RESEARCH ARTICLE

Assessment of Self-Efficacy through Pre-Incubational Activities among Female Students in Higher Education Institutions, Coimbatore District

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ABSTRACT

Entrepreneurship Education is the basic theoretical aspects of entrepreneurship within the college or university campus. Training of knowledge and skills are more important for the students to start their own business. Whereas, the major role of incubation centers is to promote innovative business within the local society through start-ups. The major role is played by Self-Efficacy that resembles as a choice of learning and knowing the self capacity of the individual to set-up a new firm. As, it is an era of innovation and advancement, students and individuals strive towards the authentication factor of choosing a career that leads to emergence of new start-ups. This research paper aims to extract the insights of the factors that can act as antecedents to assess the effectiveness of Pre-Incubational activities among female students to assess the self-efficacy as personal variable to determine the capabilities of becoming an entrepreneur. India is a powerful center for business, at the same time, job creation fails to accommodate talented minds due to increased population of the country, it is important to know the role of individual's willingness towards entrepreneurship education and pre-incubational training set-ups for the development of individual's business ideas. The purpose of the study is to examine how incubational activities plays an active role assessing the individual self-efficiency which works as a support system to create own start-up company and choose entrepreneurship as an occupation.

Keywords: Entrepreneurship Education, Incubation center, Self-efficacy, Start-ups.

INTRODUCTION

In developing countries, in the present 21st century, entrepreneurship is considered to be the most important economic driving strategy, which paves way for the development of country as well as individuals. It creates a sustainability of competitiveness and also utilize the opportunities of globalization (Schaper and Volery, 2004; Venkatachalam and Waqif, 2005). (Gorman, Halon et al. 1997; Lena and Wong 2003; Karanassios, Pazarskis et al. 2006; Banchflower, 2000) stated that, a positive relationship can be seen between entrepreneurship and economic growth in terms of job creation, firm sustainability and technological change. India is one of the largest developing country, as per 2018 data, India holds the 3rd largest start-up ecosystem in the world. It is about 50,000 start-ups. In the current scenario, Tamil Nadu acts a witness in the development of entrepreneurship, social mobility, economic growth and technology innovation, where Coimbatore District works as a central hub for emergence of new start-up opportunities among individuals through various phases. Entrepreneurship Education ii) Role of Incubation Centre's iii) Self-Efficacy. In this paper, the study seeks about, business incubators and self-efficacy together finds the effectiveness of entrepreneurial intentions among individuals. Student's entrepreneurship as a career option is increasing and this growing interest is emphasized by various researchers (Brenner, Pringle, & Greenhaus, 1991; Fleming, 1994; Kolvereid, 1996; Zellweger, Sieger, & Halter, 2011). Thus, a major impact on the attitudes of students in relation to entrepreneurship is found in university environment (Autio, Keeley, Klofsten, & Ulfstedt, 1997; Fayolle & Linan, 2004; Johannisson, 1991; In (2014 Fayolle & Linan) stated that, in different spaces of teaching, research and outreach, the university environment has adopted conceptualized learning. On the other hand, the role of business incubation centre's help individuals grow on new start-up ideas with task and outcome-based training and development. Business incubators originally started in the 1960's, but started to play an active role in supporting start-up companies those who needed advice and venture capital in late 1990's. The major role of incubation centre's is to promote the development of innovative business within local society. Self-efficacy 'spin-off' of Bandura's theory of self-efficacy, defines as, "the strength of a person's belief that he or she is capable of successfully performing various roles and tasks of an entrepreneur". According to (Urban 2010), entrepreneurial self-efficacy is a salient feature to the career decision-making process of the potential entrepreneur.

Review of Literature

Basically, entrepreneurship is a platform to recognize opportunities, formulate the business concept by identifying the resources and launch the business. To launch a new business and convert it into growth stage, certain entrepreneurial skills and competencies are required (Morris, Webb, Fu & Singhal, 2013; Mokgari & Pwaka, 2018). Entrepreneurship education has gained prominence globally and facilitated as entrepreneurship engagement (O'Cornnor, 2013). The main goal of entrepreneurship education is to encourage students to purse entrepreneurial career (Auken, 2013).As a connection to this, Higher Education Institutions impose entrepreneurship education as a learning programmes in society to improve economic development and innovation (Nabi, Wamsley, Linan, Akhtat & Neame, 2018). For students to teach the business development models and formation of a business, entrepreneurship education must be converted as a series of training courses (Bechanrd and Toulouse 1998), thus failure to purse entrepreneurial career can be prevented (Shepherde, 2000). Therefore, it is believed that, through entrepreneurship education, these requirements of skills and competences can be developed (Martin, Mcnally, & Kay, 2013; Robinson & Sexton, 1994). Simultaneously, Incubation Centre's play a vital role in shaping the induvial to start-up new business and choose entrepreneurship as a career option. Business incubators main aim is to nurture the development of new start-up's, help them to survive and grow in the process. The foremost goal of incubation centre's is to create job in a community, enhance the entrepreneurial climate, retain the business, accelerate growth of local business and diversify local economy. The National Business Incubation Association (NBIA), states that, "business incubation is a dynamic process of business enterprise and development, to accelerate successful start-up's with array of targeted resources and services". The European Commission, in its Benchmarking of Business Incubators, defines a business incubator as 'an organization that accelerates and systemizes the process of creating successful enterprise by supporting incubator space, business support services and networking opportunities, where

as (Fernandez Fernandez& Blanco Jimenez, 2011) cited that, the role of business incubation is to improve the survival and growth prospects of new start-up's. In connection to the development of start-up and entrepreneurship, self-efficacy plays an important role in creation of entrepreneurial intention research among students and individuals (Boyd & Vozikis, 1994; Chen, Greene, & Crick, 1998; Fuller, Liu, Bajaba, Narler, & Pratt, 2018; Hsuet al., 2018; Krueger & Brazeal, 1994; Naktiyok, Nur Karabey, & Caglar Gulluce, 2010; Scmutzler, Andonova, & Diaz-serrano, 2018; Zaho, sEibert & Hills, 2005). Self-efficacy is defined as, (Albert Bandura, 1977, 1982) "beliefs in one's capabilities to mobilize the motivation, cognitive resources, and courses of action needed to meet given situational demands (Wood & Bandura, 1989, p 408).Against the requirements of career path, people evaluate the capabilities for choosing the career path. Individuals engage in behaviours of more efficacious at and avoid others they know they are less competent in completing (Chen, Greene, & Crick, 1988; Rocio Fernandez-Ballesteros, diez-Nicolas, Caprara, Barbaranelli, & Bandura, 2002). The individual's behaviour is viewed through reciprocal causation among cognitive, behavioural, and environmental factors (Bandura 1977; Chen et al., 1998)that is grounded in social learning theory (Bandura 1977, 1982; Wood & Bandura 1989). As reported by (Chen et al., 1998) the perception of the self-environment can be inclusive to the changes in people's actions and in turn, the people's behaviours and actions can be changed by environment and perception of the self as well. Thus, self-efficacy, has a level of influence on individuals' "choice of activities, goal levels, persistence, and performance in a range of contexts" (Zaho, Seibert, & Hills, 2005, p.1226).

Objectives of the Study

- Assess the self-efficacy of the students with-in campus through pre-incubation activities
- Create an on-campus entrepreneurship learning centre for students

RESEARCH METHODS

The research tools are used to formulate the objectives of the study. The research design adopted using sampling technique and tools applied to satisfy the research.
Area of study- Coimbatore
Sampling Unit – female students
Sampling Size – 100
Sampling Technique – Non-probability judgmental sampling technique
Data Collection method - Primary data collection method – 100 samples were collected through questionnaire among female students. Secondary data collection method - data through articles, journals, books were collected for the study. The following are the diagrammatic representation of Operational Definitions of the concept that assess the level of self-efficacy through incubational activity.

Pre-Incubation Activity: The incubation activity within the Institutions helps the students in transforming themselves from the level of student to become an entrepreneur. The Entrepreneurship Development Cell in the Institutions helps students through pre-incubational activities by conducting regular workshops, training programs, attend seminars and workshops. It also makes students participate in business plan contest. Private incubators conduct training programs through developing knowledge on entrepreneurship create ideation stage, build the ideas and network the student for initial investments and create own start-up.

Self-Efficacy: It is the belief of an individual to create self confidence for their self functioning. Self-efficacy acts as a motivational path for the individual to work on right decision making, and personal accomplishment. Research design is exploratory in nature. The research gap identified is related to the assessment of self-efficacy of students through incubation activities which creates an entrepreneurial intention among students. Based on the sustainability, the mean score value is calculated on the role of Higher Education Institutions which includes the role of incubation centers and self-efficacy of students in attending entrepreneurial activities.

Analysis and Interpretation

Analysis is based on the variables that are considered as the key factors. It constitutes the role HEIs in considering the level of self-efficacy of students through pre-incubation activities. The mean score value of Entrepreneurship course in syllabus is **3.97**. The role HEIs in conducting curriculum course creates a higher impact among the students in developing entrepreneurship intention. To examine the level of pre-incubation activity among students, self-efficacy is also analysed. The major role is played by the staff members and mentor were the mean score value is **3.8.** the students are advised and infused with the knowledge of entrepreneurship course as curriculum and choose entrepreneurship as a career and start new firm in the future.

Findings

Assessment of self-efficacy through pre-incubational activities among female students in HEIs

Coimbatore is an active centre for the emergence and growth of medium and small-scale industries. A study was conducted among female students from various colleges to know the level of self-efficacy of becoming an entrepreneur through incubation training and start-up ideas. With the up gradations of technology, new start-up ideas generate through various platforms such as, entrepreneurship education where the universities and colleges work as a basic platform for boosting the self-efficacy among students through entrepreneurship education as curriculum. The Indian business background is characterized by two incubation drives, namely, technology centre's and business incubation with the purpose of inspiring financially viable development and sustainability. Business incubation centre's are participants in the growth of new start-ups. There are private incubators and technology incubation centre's created by the educational institutions. Higher Education Institutions provide students with basic learnings of the need for entrepreneurial career intentions, goal oriented, develop self-efficacy towards own job creation.

The role of business incubators is to produce competent entrepreneurs for economic appraisal. (Seoane, 2014) mentioned on, a study conducted in Spain, Business Incubator in Galicia, established a positive relationship on incubators assisting new start-up's by influencing training and gender in entrepreneurship. The improvement and performance, allocation of entrepreneurship training programs are model accelerator tools by incubation organizations (Al-Mubaraki & Hamad). These business incubation centre's are focused on scarce resources, provide relevant information and to create an atmosphere to encourage learning; the managers or incubators' approaches to be changed from the act managerial role to mentors for encouraging incubates. Recruitment practices to include more holistic appreciation of potential incubates, as contribution is made towards learning community as well as assessment in the business plans.

Recent studies yield support for the role of entrepreneurial self-efficacy in entrepreneurial intentions and mainly, Self-efficacy is considered to be a mediator between other variables (BarNir, Watson, & Hutchins, 2011; Chen & He, 2011; Fuller, Liu, Bajaba, Marler, & Pratt, 2018; Piperopoulos & Dimov, 2015; Schmutzler, Andonova & Diaz-serrano, 2018). Self-efficacy construct is considered as a multidimensional factor by (Chen & He, 2011), it is constructed by, opportunity identification, relationship, managerial efficacies. Higher self-efficacy teaching has a stronger effect on student entrepreneurial intention through theoretical entrepreneurship courses (Piperopoulos & Dimov, 2015). Self-efficacy plays a moderation role in relationship between socio-cultural environments, individualism and entrepreneurial intention (Schmutzler et al., 2018). It has a stronger effect on the perseverance of individual to fit themselves with entrepreneurship. This efficacy is influenced by factors such as entrepreneurship education, culture, personal factors, etc.

CONCLUSION

A suggestive model is created for the HEIs to play an efficient role in developing entrepreneurship intention and create a mind-set among students in developing themselves for career advancement. The main objective of the study is understand the role taken by HEIs to create awareness and build self-efficacy among students at various stages and sensitizes their entrepreneurial path. Pre-incubation activities focus on building the ideation stage which is said to be the proof-of concept within the available possibilities. The students self-confidence is made higher by the opportunities provided by HEIs. It works as a platform to express the innovation idea of the students to get mpral and technical support in creating a new business model. The entrepreneurship course plays a major role which includes seminars, workshops, innovation centres mentoring to comprehend the feasible idea into a business start-up. Therefore, On-Campus entrepreneurial programmes enhance the development of self-efficacy among students in HEIs.

REFERENCES

1. Veronika Bikse and Inese Lusena-Ezera Liepaja University, Management Sciences Institute, Latvia Baiba Rivza Latvia University of Agriculture, Latvia Tatjana Volkova BA School of Business and Finance, Latvia "The Transformation of Traditional Universities into Entrepreneurial Universities to Ensure Sustainable Higher Education", *Journal of Teacher Education for Sustainability, vol. 18, no. 2, pp. 75ñ88, 2016*
2. Étienne St-Jean & Cécile Fonrouge (2020) Comment developer l'auto-efficacité entrepreneuriale? Étude auprès d'étudiants universitaires, Journal of Small Business & Entrepreneurship, 32:4, 355-377, DOI: 10.1080/08276331.2018.1560842
3. Panagiotis Piperopoulos & Dimo Dimov (2015) Burst Bubbles or Build Steam? Entrepreneurship Education, Entrepreneurial Self-Efficacy, and Entrepreneurial Intentions, Journal of Small Business Management, 53:4, 970-985, DOI: 10.1111/jsbm.12116
4. Moustafa Elnadi, Mohamed Hani Gheith, Entrepreneurial ecosystem, entrepreneurial self-efficacy, and entrepreneurial intention in higher education: Evidence from Saudi Arabia, The International Journal of Management Education, Volume 19, Issue 1, 2021, 100458, ISSN 1472-8117
5. Gustavo Hermínio Salati Marcondes de Moraes Edson Sadao Iizuka Matheus Pedro Effects of Entrepreneurial Characteristics and University Environment on Entrepreneurial Intention, Artigo • Rev. adm. contemp. 22 (2) • Mar-Apr 2018 https://doi.org/10.1590/1982-7849rac2018170133

Table 1: Analysis of Pre-Incubation Activity

Pre-Incubation Activity	Mean Score
Entrepreneurship course in syllabus	**3.97**
Entrepreneurship Development Cell (EDC)	3.93
Develop entrepreneurial skills	3.87

Table 2: Analysis of Self-Efficacy

Self-Efficacy	Mean Score
Mentoring and advice regarding entrepreneurship	3.81
Enhance student confidence level by redefining idea	3.79
Incubation and acceleration programs	3.76

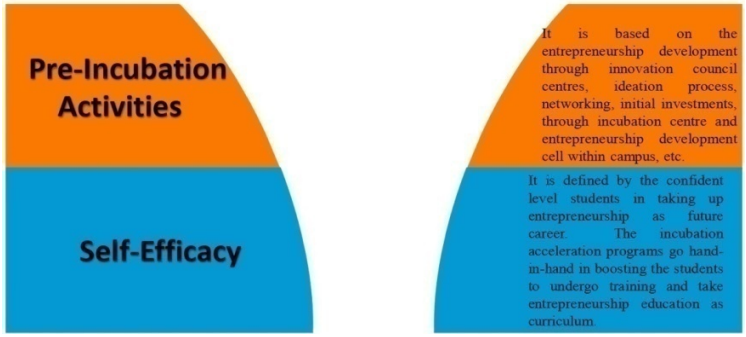

Figure 1: Operational Definitions

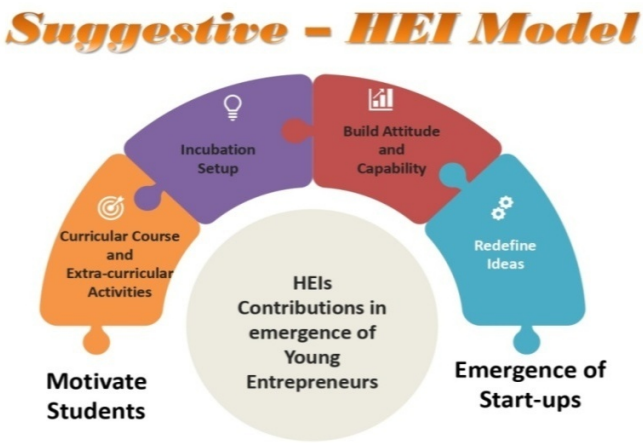

Figure 2: Suggestive HEI Model

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REVIEW ARTICLE

Bhringraj: A Pharmaceutical Treasure Trove

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ABSTRACT

Eclipta alba, commonly known as Bhringraj, a plant known throughout Indian history for its various medicinal uses. Different regions use the plant extracts for a variety of cures, amongst which the commonly known properties of its extract are anti-hepatotoxic, anti-diabetic, anti-inflammatory and hair rejuvenator. This review further explores both the well-known as well as lesser-known properties of Bhringraj, such as anti-cancer, anti-oxidant, neuroprotective, anti-fungal, anti-bacterial, cytotoxic, anti-hyperlipidaemic, anti-helminthic, anti-venom and analgesic activities. It also emphasizes the compounds or chemicals extracted from the plant parts and their properties with a short pharmacological profile. Though some of the properties of Bhringraj have been explored for pharmacological benefits, there is still a long way to go for its full potential to be exploited for human welfare.

Keywords: Ayurveda, Medicinal herbs, Bhringraj, *Eclipta alba, Eclipta prostrata*, Asteraceae (Sunflower) family, False Daisy

INTRODUCTION

For years, humans have been trying to find remedies to diseases and disorders from plant extracts. For centuries, work done by our ancestors has resulted in the discovery of these cures and thus, India has emerged as a large repository of herbs and plants which possess medicinal value, and they have been exploited since the ancient era. Treatments for several diseases have been offered through remedies mentioned by Ayurveda since olden times. Many pharmaceutical industries have recently shifted their focus to produce various medicines from the extracts of plants/herbs. Even though medicinal herbs are beneficial, allopathy is chosen as they have a comparatively faster mode of action which is responsible for curing the

problem of interest in comparison to medicinal herbs. Bhringraj is a medicinal plant widely used in the eastern part of India. *Eclipta alba (L.) Hassk* (synonym *Eclipta prostrata*) is a herb that grows annually. It can be erect or prostrate and belongs to the Asteraceae (Sunflower) family, also known as the Compositae. It is known as 'Bhringraj' in Sanskrit, 'Bhangra' in Hindi, 'Keshatta' in Bengali, 'Maaka' in Marathi, and by a few other names in different parts of India. *Eclipta alba*, commonly known as False Daisy, is known to grow in warm and temperate to tropical regions upto the height of 1.0-2.0 feet and is usually located near a water source i.e. a moist place. It grows in tropical regions worldwide such as in countries like India, Bangladesh, Nepal, China, Pakistan, Indonesia, Thailand (South-East Asia) and Brazil. The leaves are opposite sessile and lance-shaped and are 2.0-6.2 cm long and 1.5-1.9 cm wide. The stem is cylindrical or flat, rough due to the presence of appressed white hairs; nodes are distinct and greenish or occasionally brownish. The root is taproot and is greyish and is not very hairy. The plant's growth is supported on various soil types, which ranges from sandy to clay soil and a number of other areas such as waterlogged low regions, wastelands which are damp, side of the roads, paddy fields, as well as fields of other crops, especially regions with warm climate. In South-east Asia, especially in India, the dried whole plant, the extract, and its paste is used in traditional medicine especially as an Ayurvedic proprietary medicine.

In Ayurveda, Bhringraj has been in use since ancient times and it classifies Bhringraj as 'Shweta', 'Peet', and 'Neel', which are white, yellow and blue flowers respectively. It is harvested for its numerous therapeutic properties like an astringent, depurative, emetic, purgative, nervine tonic, catarrhal, febrifuge, ophthalmic, styptic, tonic, anti-nociceptive, anti-leprotic, anti-hemorrhagic, anti-myotoxic, anti-hyperlipidemic, anti-diabetic, hepatoprotective, diuretic, hypotensive, nootropic, anti-venom, ovicidal and spasmogenic. Bhringraj is a traditional plant, with plenty of ayurvedic uses. Bhringraj tastes tart, sharp, and bare. The Ayurvedic encyclopedia i.e. 'Raj Nighantu' explains the importance of this plant in great detail. The ancient use of Bhringraj in Raj Nighantu includes Kusthahara (Treat skin infections/diseases), Kesharanjaka (Hair protector/vitaliser), Raktapitta Shothahara (Treat blood disorders; Anti-inflammatory), Jantujit (Cure's worm trichinosis), Rasayana (Anti-ageing and Anti-cancerous), Pachana (Cures digestive problems), Kasahara (Cures cough and lung-related problems) [1].

One of the most known properties of *Eclipta alba* is its hepatoprotective or anti- hepatotoxic properties. *Eclipta alba* (L.) Hassk. has been widely used in India for the traditional treatment of liver disorders [2]. The coumestans- wedelolactone and dimethyl wedelolactone, isolated from this plant have been reported to exhibit anti-hepatotoxic activity in assays employing CCl_4, galactosamine and phalloidin induced cytotoxicity in rat hepatocytes [3]. *Eclipta alba* powder has been found to counteract an increase in liver weight, hepatic lipid peroxidation, liver γ-glutamyl transpeptidase, serum alanine transferase, serum alkaline phosphatase and serum albumin to globulin ratio induced in rats (*in vivo*) by CCl_4. The tincture of the plant is used for liver and kidney problems and it is also reported to have therapeutic potential against cardiovascular disorders [4]. In recent years, microbial infections have increased to a great extent and antibiotic resistance has become an ever-increasing therapeutic problem [5]. The aqueous and alcoholic extracts of the plant are proved to confer neuropharmacological activity [6]. Screening of anti-fungal properties have also been studied in some fungal strains like *Candida tropicalis*, *Rhodotorula glutinis*, and *Candida albicans* [7]. The plant *Eclipta alba,* performs the crucial function of mediator of the exogenous type, to stimulate proliferation of follicular keratinocyte and at the same time retard the terminal differentiation with the help of downregulation of the expression of TGF-β1. Thus, it can be used in the treatment of certain types of alopecia [8]. Luteolin, present in the Bhringraj plant extract, plays a role in preventing epileptic seizures [9, 10]. This plant has also been mentioned as a nervous tonic [11].

Pharmacological Properties: The pharmacological activities of Bhringraj were explored from different sources such as research papers, web resources, databases and books. The properties and activities were studied in depth to know the different role of the phytochemical constituents of the plants with respect to the respective pharmacological properties and are classified below:

Anti-breast cancer activity: Alcoholic extract of *Ecilpta alba* (AEEA) has been used to test the anti-breast cancer activity on human as well as mouse cell lines. Treatment of breast cancer cells with AEEA causes apoptosis. Treatment of AEEA also causes plasma membrane disintegration, chromatin condensation, and apoptosis-mediated cell death [12]. AEEA causes a decrease in mitochondrial membrane potential, which subsequently initiates a cascade leading to apoptosis [13]. This activity of AEEA helps in the reduction of the side effects observed during therapy for cancer. An innovative approach using phytochemical luteolin, isolated from *Eclipta alba,* with Zinc oxide nanoparticles was evaluated as ZnO nanoparticle-guided delivery of luteolin to promote anti-tumorigenic activity [14]. Thus, looking at these properties of the AEEA, it may be a novel anti-cancer medicine and can be used as an alternative source of medicine for breast cancer treatment as well as cancer of other types.

Anti-ulcer and anti-colon cancer activities: The ethanolic extract of *Eclipta alba* showed potent anti-ulcer activity in a dose dependent manner and significantly reduced ulcerative lesions as well as attenuation of lipid peroxidation in a rat model [15]. In another study, methanolic extract of *Eclipta alba* showed pronounced reduction in gastric ulcers and inflammation in rats treated with aspirin to induce ulcers [16]. Crude *Eclipta alba* methanolic extract showed a targeted anti-cancer effect against colon cancer cell lines HCT-116 using MTT assay. The methanolic extract of *Eclipta alba* bears anti-colon cancer compounds which would be worth investigating in the future for possible therapeutic development [17].

Anti-oxidant activity: Oxidative stress leads to the release of free radicals which affects numerous disorders such as neurodegenerative diseases, cancer, atherosclerosis, and angina pectoris. Anti-oxidants due to their scavenging activity are useful for the management of these diseases [18]. *Eclipta alba* is a rich natural source of anti-oxidants, such as phenolic acids, flavonoids, and terpenoids, and thereby capable of abolishing oxidative stress [19]. Phenolics act as a reducing agent, singlet oxygen quenchers, hydrogen donors, and metallic chelating potential. *Eclipta alba* treatment significantly reduces TBARS, H_2O_2, and nitric oxide levels and restores anti-oxidant defense [20].

Neuroprotective activity: Amongst other properties of the plant, *Eclipta alba* shows the property of neuroprotection as well. Its treatment in the stressed animals showed significantly reduced DNA damage. It improves anti-oxidant enzyme levels and reduces brain oedema [21]. Pathogenic development of Parkinson's disease involves degeneration of dopaminergic neurons and is preceded by an overwhelmed oxidative stress condition and neuroinflammation. The alcoholic extract of *Eclipta alba* helped in providing protection against MPP^+ induced oxidative damage in SH-SY5Y cells. This signifies that the phytochemical(s) present in *Eclipta alba* provides an effective natural therapeutic solution for Parkinson's disease [22]. *Eclipta alba* is known to show a reduction in tension and cholesterol in one's system. The effect of hypotension is a decrease in systemic blood pressure, and at the same time reduction in cerebral oedema [23]. *Eclipta prostrata* enhances the memory function in a rat's brain against dementia, by increasing acetylcholine levels through inhibition of the activity of the enzyme acetylcholinesterase [24]. Luteolin and apigenin are two important compounds present in the extract which are known to possess several neuroprotective activities. These compounds have beneficial effects in the treatment of epilepsy, autism spectrum disorders, Alzheimer's disease, Parkinson's disease, diabetes-associated cognitive disease, traumatic brain injury, and multiple sclerosis [25].

Anti-inflammatory activity: Inflammation is a physical condition which is observed by swelling, redness, pain, heat and at times a loss of function as a result of infection or injury. Inflammatory mediators like complement proteins, histamine, kinins, prostaglandins and pro-inflammatory cytokines have been suggested to play a role in the mechanism of inflammation in rats [26]. Some of these mediators are considered to be inhibited by the methanolic extract of leaves of *Eclipta prostrata*. The anti-inflammatory activity exhibited by methanolic extract of whole plants of *Eclipta prostrata* has shown similar effects as that of the standard

drugs such as indomethacin and cyproheptadine. The aqueous extract of *Eclipta prostrata* seeds showed inhibition of protein (egg albumin) denaturation in a dose dependent manner with an IC50 value of 1710 μg/ml with pure diclofenac sodium as a standard reference drug [27]. *In vitro* egg albumin assay is a standard technique for measuring anti-inflammatory activity and even though its effectiveness was lower than the standard drug which is a NSAID, the potential of *Eclipta prostrata* component(s) as a safe anti-inflammatory remedy cannot be ignored. The methanolic extracts of *Eclipta prostrata* leaves have proven to show the anti-oedematous property during the first phase of oedema development by inducing an inhibitory effect on the release of active pain substances such as histamine, serotonin, polypeptides, or prostaglandins [28]. Wedelolactone, an important constituent derived from the plant, is thought to suppress inflammation by inhibiting infiltration of neutrophils along with decreased MPO activity, thus regulating anti-inflammatory responses of fungal keratitis in mice. It has also proven to decrease the maturation of the pro-inflammatory IL-1β which is one of the pivotal mediators of host immune response to inflammatory stimulation [29].

Anti-diabetic activities: The extract of *Eclipta alba* tends to have components that are biologically active and thus provide stability to the nanoparticles of gold [30]. For the purpose of green synthesis of gold nanoparticles (AuNPs), methanolic extract of *Eclipta alba* was mixed with auric chloride in the proportion 2:8, and heated at 80℃. These AuNPs produced from *Eclipta alba* extract have exhibited anti-diabetic, anti-oxidant as well as anti-bacterial properties. The anti-oxidant property is concentration dependent and is weaker in comparison to anti-oxidant action of ascorbic acid. The interaction of the charges on the surface of the bacteria and *Eclipta alba* extracted AuNPs (EA-AuNPs), may be responsible for the anti-bacterial activity. But the main outcome of this study was the fact that *Eclipta alba* AuNPs reduced apoptotic cell death in STZ-treated cells, indicating the protection of pancreatic beta cells from dying. The down-regulation of Bcl-2, up-regulation of Bax and modulation of NF-κB could be a possible mechanism for the anti-apoptotic activity of EA-AuNPs and thus its role as a potent anti-diabetic agent [31].

Anti-fungal activity: The unregulated usage of synthetic/chemical fungicides for disease management has many environmental imputations like drug resistance in the target pathogens and poses pollution issues. *Eclipta alba* shows potential anti-fungal properties. It has been tested to be effective against plant fungi *in vitro* as well as *in vivo* conditions, for e.g. Sorghum grain mould pathogens such as *Fusarium thapsinum*, *Alternaria alternata*, *Epicoccum sorghinum*, and *Curvularia lunata* [32].

Anti-bacterial activity: Currently, many pharmaceutically important secondary metabolites are isolated from herbal plants or trees as their artificial synthesis is not economically feasible. Natural products obtained from plants serve as a major source of medicine and about 1/4th of total drugs found today come from plant origins. The methanolic and butanolic extract of *Eclipta alba* shows a very effective anti-microbial activity against various species of bacteria and fungi [33]. This indicates the potential anti-bacterial nature of this plant. The anti-bacterial compounds present in this plant may serve as an affordable and new source for the treatment of infectious disease [34]. *Eclipta alba* has been tested against *Klebsiella pneumoniae* in an *in silico* docking study using the software application 'Biovia Discovery Studio'. The results indicated that the glutamic acid present in the extract as a phytochemical and used here interacts with the bacterial enzyme glycerol dehydrogenase to disrupt the life cycle of the organism. Therefore, major phytochemicals evaluated for the anti-bacterial activity against *Klebsiella pneumoniae in silico* were glutamic acid, phenylalanine, luteolin, cystine and apigenin with glutamic acid showing the most effective interaction [35].

Cytotoxic activity: Herbal medicine represents an important part of traditional medicine. For the treatment of several physical, physiological and mental problems, herbal medicines are used extensively. *Eclipta prostrata* holds an excellent reputation of having been used as an agent of medicine. The cytotoxicity screening is a standard marker for anti-cancer activity as the anti-tumour and anti-proliferative presence of

plant extract can be evaluated by it. In a major study, the carbon tetrachloride soluble fraction of methanolic extract of *Eclipta prostrata* showed the highest degree of cytotoxicity having LC50 of 1.318 µg/ml. It proves that *Eclipta prostrata* is a potent cytotoxic drug and can be used in cancer therapeutics which will be available at much cheaper costs and will be much more safe than the chemotherapeutic drugs which are currently utilized for the purpose of the treatment of cancer [36].

Anti-hyperlipidaemic activity: The mainstream Ayurvedic practice in India uses *Eclipta prostrata* to treat hyperlipidaemia. The value of the treatment was proved by research conducted on Albino rats using the alcoholic extract of *Eclipta prostrata*. The dose of 150 mg/kg & 200 mg/kg body weight of the rats decreased the total triglycerides and other lipid levels in serum, liver, and heart. The control subject group was investigated with standard drugs (clofibrate/guggul); the results obtained with the rats treated with *Eclipta prostrata* extract were similar to that of the control subject group. This result supports the hypolipidemic activity of *Eclipta prostrata* and its use for the treatment of anti-hyperlipidaemia [37].

Anti-hepatotoxic activity: *Eclipta alba* when extracted in ethyl acetate as a solvent, has shown exceptional hepatoprotective effects, against CCl_4 and D-GaIN induced liver damage and also against phalloidin which is known to be a strong poison. The C_7-OCH_3-substituted wedelolactone was more active against CCl4-cytotoxicity than dimethyl wedelolactone. However, these effects were dependent on the concentration of the dose and the phyto-components responsible for the hepatoprotective activity includes apigenin, luteolin and wedelolactone [38]. In this regard, hepatoprotective effects of *Eclipta alba* could be compared to the well-known hepatoprotective effects of *Silybum marianum* plant [39]. Similarly, the ethanolic extract also showed good and dose-dependent efficacy against the hexobarbitone induced narcosis, zoxazolamine-induced paralysis and CCl_4 induced liver damage [40]. In another study, the hepatoprotective effects of *Eclipta alba* at subcellular levels in rats revealed that the hepatic lysosomes were protected due to the action of the phytochemical components of the extract on the enzymes of lysosome and not of mitochondria, thus protecting hepatocytes, which in turn provided protection to the liver [41].

One of the causes of liver cirrhosis is Non-Alcoholic Fatty Liver Disease (NAFLD), which involves deposition of fats in the liver despite not consuming alcohol, and may become one of the major chronic liver disorders all over the world [42]. Considering the hepatoprotective properties of the *Eclipta alba,* the extract of *Eclipta alba* was tested for its effect on NAFLD. The disease was induced in rats through a high fat diet for 8 weeks straight and later fed *Eclipta alba* extracts for 1, 2 and 3 weeks in different groups. The rats group fed with *Eclipta alba* extract for 3 weeks straight, showed the best results, resulting in complete regeneration of the liver, thus portraying impressive hepatoprotective properties of *Eclipta alba* in combating NAFLD [43]. Looking at the significant medicinal properties of this plant, the need is to find out the correct procedure of micropropagation of *Eclipta alba*. The most important component of *Eclipta alba*, namely wedelolactone, is produced when cytokinin is supplemented abundantly and also the basal MS media should be supplemented with 2.4 µM for the process of micropropagation as a whole [44].

Anti-helmintic activity: *Eclipta prostrata* have been used as an anti-helmintic drug in Africa by traditional medicine. The whole plant extract of *Eclipta prostrata* in methanol is one of the most effective natural anti-helmintic drug [45].

Anti-venom activity: *Eclipta alba* contributes majorly to the property of anti-venom, and the three main components of action are due to the presence of stigmasterol, sitosterol, and wedelolactone [46]. This activity has been proved to be effective against venoms of species such as *Bothrops jararaca, Bothrops jararacussu*, *Lachesis muta* [46] and *C. durissus terrificus* [47]. The components of the plant utilize the mechanism such as anti-phospholipase A_2 activity and anti-myotoxic for inhibitory purposes [46, 47].

Coumestans, an important aromatic group of chemicals present in Bhringraj as well as other plants, possess potent anti-venom activity [48,49]. In another study, both native and genetically altered *Eclipta alba* were efficient in inhibiting snake venom phospholipase A2 activity against a variety of species and contained coumestans in different parts of the plant [50].

Analgesic property: Acid-alcoholic digested extract of *Eclipta alba* when orally administered to 25 Swiss albino rats showed similar analgesic properties as that of Aspirin and Codeine. The ethanolic extract of *Eclipta alba* possesses good analgesic activity if doses are administered at 250 mg/kg and 500 mg/kg orally. Also the chloroform soluble portion, which is total alkaloids, showed good analgesic activity at a dosage of 150 mg/kg. These extracts are effective in both the central as well as peripheral models of pain [51].

Hair rejuvenation: There have been claims of *Eclipta alba* having excellent hair growth properties, but not many clinical trials have been done on the same. When the action of petroleum ether and ethanolic extract of the plant was tested on mice for the purpose of observing hair growth properties in terms of quality, quantity and the time taken for the growth to occur at the same time, the fraction obtained in petroleum ether showed excellent results in comparison to minoxidil (standard FDA approved drug for alopecia). On the other hand, ethanolic extract did not show such effects. Since the ether fraction contains β-sitosterol and wedelolactone, these can be considered to be the main factors for quality hair growth [52].

CONCLUSION

Bhringraj is only known for its role as a hair growth rejuvenator and protector of the liver. But, in this review, various other important pharmacological activities of this plant have been reported. They include anti-cancer, anti-oxidant, neuroprotective, anti-inflammatory, anti-diabetic, anti-fungal, anti-bacterial, cytotoxic, anti-hyperlipidaemia, anti-hepatotoxic, anti-helmintic, anti-venom and analgesic activities. Wedelolactone, a major constituent from this plant is considered to have a central role for quite a few of these properties. Despite possessing all these properties, little clinical research has been done on Bhringraj and/or its extracts. With such a broad spectrum of medicinal properties, the significance of Bhringraj can be thought to be of greater value to modern medicine and therefore warrants in-depth basic and clinical studies in order to exploit its wide-spectrum properties.

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REFERENCES

1. Sarup L. The Nighantu and the Nirukta: The Oldest Indian Treatise on Etymology, Philology and Semantics. Motilal Banarsidass Publ.; 1998.
2. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research.1956. p.55.
3. Chandra T. Effect of Eclipta alba on Inflammation and Liver Injury. Fitoterapia. 1978;58:23-31.
4. Mithun NM, Shashidhara S, Vivek Kumar R. Eclipta alba (L.) A Review of its Phytochemical and Pharmacological Profile. Pharmacologyonline 2011;1(1):345-357.
5. Austin DJ, Kristinsson KG, Anderson RM. The Relationship between the Volume of Antimicrobial Consumption

in Human Communities and the Frequency of Resistance. Proceedings of the National Academy of Sciences. 1999 ;96(3):1152-1156.

6. Thakur VD, Mengi SA. Neuropharmacological Profile of *Eclipta alba* (Linn.) Hassk. Journal of Ethnopharmacology. 2005 Oct 31;102(1):23-31.
7. Venkatesan S, Ravi R. Antifungal activity of *Eclipta alba*. Indian Journal of Pharmaceutical Sciences. 2004;66(1):97.
8. Begum S, Lee MR, Gu LJ, Hossain J, Sung CK. Exogenous Stimulation with *Eclipta alba* Promotes Hair Matrix Keratinocyte Proliferation and Downregulates TGF-β1 expression in nude mice. International Journal of Molecular Medicine. 2015;35(2):496-502.
9. Tambe R, Patil A, Jain P, Sancheti J, Somani G, Sathaye S. Assessment of Luteolin Isolated from Eclipta alba Leaves in Animal Models of Epilepsy. Pharmaceutical Biology. 2017;55(1):264-268.
10. Shaikh MF, Tan KN, Borges K. Anti-convulsant Screening of Luteolin in four mouse Seizure models. Neuroscience letters 2013;550:195-199.
11. Satyavati GV, Raina MK, Sharma M. Medicinal plants of India. Indian Council of Medical Research; 1987(Vol. 2).
12. Yadav NK, Arya RK, Dev K, Sharma C, Hossain Z, Meena S, Arya KR, Gayen JR, Datta D, Singh RK. Alcoholic Extract of *Eclipta alba* shows *in vitro* Antioxidant and Anticancer Activity without Exhibiting Toxicological Effects. Oxidative Medicine and Cellular Longevity 2017 Jan 31;2017.
13. Perry SW, Norman JP, Barbieri J, Brown EB, Gelbard HA. Mitochondrial Membrane Potential Probes and the Proton Gradient: a practical usage guide. Biotechniques 2011;50(2):98-115.
14. Kollur SP, Prasad SK, Pradeep S, Veerapur R, Patil SS, Amachawadi RG, Lamraoui G, Al-Kheraif AA, Elgorban AM, Syed A, Shivamallu C. Luteolin-fabricated ZnO Nanostructures Showed PLK-1 Mediated anti-breast Cancer Activity. Biomolecules 2021;11(3):385.
15. Kumar SP, Kumar BS, Chandana VR, Vijaykumar M, Ojha SK, and Bavani ME. Antisecretory and antiulcer activities of *Eclipta alba* Linn. In rats. Proceedings of the 5th World Ayurveda Congress. 2012. Oral Presentation, Abstract No. OA01.03.
16. Banerjee A, Shrivastava N, Kothari A, Padh H and Nivsarkar M. Antiulcer activity of methanol extract of *Eclipta alba*. Indian Journal of Pharmaceutical Sciences. 2005. 67 (2):165–168.
17. Kumar Nelson V, Sahoo NK, Sahu M, Hara Sudhan H, Pullaiah CP, Muralikrishna KS. In vitro anticancer activity of *Eclipta alba* whole plant extract on colon cancer cell HCT-116. BMC complementary medicine and therapies. 2020 ;20(355):2-8.
18. Castro, L. and Freeman, B.A. Reactive oxygen species in human health and disease. Nutrition (Burbank, Los Angeles County, Calif.), 2001.17(2), 161-165.
19. Lee KY, Ha NR, Kim TB, Kim YC, Sung SH. Characterization of Triterpenoids, Flavonoids and Phenolic acids in *Eclipta prostrata* by High-performance liquid Chromatography/diode-array Detector/electrospray Ionization with Multi-stage Tandem Mass Spectroscopy. Natural Product Sciences. 2010;16(3):164-168.
20. Potterat O. Antioxidants and Free Radical Scavengers of Natural Origin. Current Organic Chemistry. 1997 ;1(4):415-440.
21. Vidyashree, H.M., Malathi, S., Rajan, R. And Devi, R.S.Neuroprotective role of herb *Eclipta alba* linn. On Acute and Chronic unpredictable Stress-induced Behavioral, Biochemical, and Neurotransmitter Alteration in Wistar male Albino Rat Brain. International Journal of Pharmaceutical Sciences and Research, 2018.9(6), 2244-2257.
22. Bhatia G, Singh J, Nehru B. Neuroprotective Effects of Hydro-alcoholic extract of *Eclipta alba* against 1-methyl-4-phenylpyridinium-Induced *in vitro* and *in vivo* models of Parkinson's disease. Environmental Science and Pollution Research. 2021;28(8):9390-9406.
23. Rangineni V, Sharada D, Saxena S. Diuretic, hypotensive, and hypocholesterolemic effects of *Eclipta alba* in mild hypertensive subjects: a pilot study. Journal of medicinal food. 2007;10(1):143-148.
24. Kim DI, Lee SH, Hong JH, Lillehoj HS, Park HJ, Rhie SG, Lee GS. The Butanol Fraction of *Eclipta prostrata* (Linn) Increases the formation of brain acetylcholine and decreases oxidative stress in the brain and serum of cesarean-derived rats. Nutrition Research. 2010;30(8):579-584.
25. Nabavi SF, Braidy N, Gortzi O, Sobarzo-Sanchez E, Daglia M, Skalicka-Woźniak K, Nabavi SM. Luteolin as an Anti-inflammatory and Neuroprotective agent: A brief review. Brain Research Bulletin 2015;119:1-1.

26. Di Rosa ML, Giroud JP, Willoughby DA. Studies of the Mediators of the Acute Inflammatory Response Induced in Rats in different sites by Carrageenan and Turpentine. The Journal of Pathology. 1971;104(1):15-29.
27. Kannadas S, Pathirana RN, Ratnasooriya WD. *In-vitro* Investigation of Anti-inflammatory Activity and Evaluation of Phytochemical Profile of *Eclipta prostrata* (L.). Journal of Pharmacognosy and Phytochemistry. 2020;9(2):831-835.
28. Arunachalam G, Subramanian N, Pazhani GP, Ravich V. Anti-Inflammatory Activity of Methanolic Extract of *Eclipta prostrata* L (Astearaceae). African Journal of Pharmacy and Pharmacology. 2009;3(3):097-100.
29. Cheng M, Lin J, Li C, Zhao W, Yang H, Lv L, Che C. Wedelolactone suppresses IL-1β Maturation and Neutrophil Infiltration in *Aspergillus fumigatus* Keratitis. International Immunopharmacology 2019;73:17-22.
30. Bhalerao SA, Verma DR, Teli NC, Murukate VR. Eclipta alba (L): AN OVERVIEW. International Journal of Bioassays. 2013;2(11):1443-1447.
31. Vijayakumar S, Vinayagam R, Anand MA, Venkatachalam K, Saravanakumar K, Wang MH, Gothandam KM, David E. Green Synthesis of Gold Nanoparticle using *Eclipta alba* and its Anti-diabetic activities through Regulation of Bcl-2 Expression in Pancreatic cell line. Journal of Drug Delivery Science and Technology 2020;58:101786.
32. Sollepura Boregowda R, Murali N, C Udayashankar A, R Niranjana S, S Lund O, S Prakash H. Antifungal Activity of *Eclipta Alba* Metabolites against Sorghum Pathogens Plants. 2019;8(3):72-87.
33. Bakht J, Islam A, Shafi M. Antimicrobial Potential of *Eclipta Alba* by Well Diffusion Method. Pak. J. Bot. 2011;43:161-166.
34. Bakht J, Islam A, Ali H, Tayyab M, Shafi M. Antimicrobial Potentials of *Eclipta alba* by Disc Diffusion Method. African Journal of Biotechnology. 2011;10(39):7658-7667.
35. Tripathy D, Adhikari C, Pandey M, Bhattacharayay D. Bhringraj Derived Phytochemicals against Pneumonia. Journal of Pharmaceutical Research International. 2020 :93-96.
36. Rahman MS, Rashid MA. Antimicrobial Activity and Cytotoxicity of *Eclipta prostrata*. Oriental Pharmacy and Experimental Medicine. 2008;8(1):47-52.
37. Kumari CS, Govindasamy S, Sukumar E. Lipid lowering activity of *Eclipta prostrata* in experimental hyperlipidemia. Journal of Ethnopharmacology. 2006;105(3):332-335.
38. Wagner H, Geyer B, Kiso Y, Hikino H, Rao GS. Coumestans as the Main Active Principles of the Liver Drugs *Eclipta alba* and Wedelia Calendulacea1. Planta Medica. 1986;52(05):370-374
39. Hikino H, Kiso Y, Wagner H, Fiebig M. Antihepatotoxic actions of Flavonolignans from *Silybum marianum* Fruits. Planta Medica. 1984;50(03):248-250.
40. Singh B, Saxena AK, Chandan BK, Agarwal SG, Bhatia MS, Anand KK. Hepatoprotective Effect of Ethanolic extract of *Eclipta alba* on Experimental Liver Damage in Rats and Mice. Phytotherapy Research. 1993;7(2):154-158.
41. Saxena AK, Singh B, Anand KK. Hepatoprotective Effects of *Eclipta Alba* on Subcellular Levels in Rats. Journal of Ethnopharmacology. 1993 ;40(3):155-161
42. Neuschwander-Tetri BA. Non-alcoholic fatty liver disease. BMC medicine. 2017;15(1):1-6.
43. Naik KS, Gurushanthaiah M, Kavimani M, Prabhu K, Lokanadham S. Hepatoprotective Role of *Eclipta alba* against High Fatty Diet Treated Experimental Models–A Histopathological Study. Maedica. 2018;13(3):21-27.
44. Franca SC, Bertoni BW, Pereira AM. Antihepatotoxic agent in Micropropagated Plantlets of *Eclipta alba*. Plant Cell, Tissue and Organ Culture. 1995;40(3):297-299.
45. Sirama V, Kokwaro J, Owuor B, Yusuf A. *In-vitro* Anti-helmintic Bioactivity study of *Eclipta prostrata* l.(whole plant) using adult *Haemonchus Contortus* worms: A case study of Migori County, Kenya.
46. Melo PA, Do Nascimento MC, Mors WB, Suarez-Kurtz G. Inhibition of the Myotoxic and Hemorrhagic activities of Crotalid Venoms by *Eclipta prostrata* (Asteraceae) extracts and constituents. Toxicon. 1994;32(5):595-603.
47. Mors WB, Do Nascimento MC, Parente J, Da Silva MH, Melo PA, Suarez-Kurtz G. Neutralization of Lethal and Myotoxic activities of South American Rattlesnake Venom by Extracts and Constituents of the plant *Eclipta prostrata* (Asteraceae). Toxicon. 1989 ;27(9):1003-1009.
48. Soares AM, Ticli FK, Marcussi S, Lourenco MV, Januario AH, Sampaio SV et al . Medicinal plants with inhibitory properties against snake venoms. Curr Med Chem 2005;12:2625–2641.

49. Mors WB. Plants against Snake-bites. Memórias do Instituto Oswaldo Cruz. 1991 Jan 1;86:193-193.
50. Diogo LC, Fernandes RS, Marcussi S, Menaldo DL, Roberto PG, Matrangulo PV. Inhibition of snake venoms and phospholipases A(2) by extracts from native and genetically modified *Eclipta alba*: isolation of active coumestans. Basic Clin Pharmacol Toxicol. 2009, 104: 293-299.
51. Sawant M, Isaac JC, Narayanan S. Analgesic studies on total alkaloids and alcohol extracts of *Eclipta alba* (Linn.) Hassk. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2004;18(2):111-113.
52. Roy RK, Thakur M, Dixit VK. Hair Growth Promoting activity of *Eclipta alba* in male Albino rats. Archives of dermatological research. 2008;300(7):357-364.

Fig 1. Shweta Bhringraj (*Eclipta alba*), growing in its natural habitat of damp wastelands.

Fig 2. Bhringraj with greyish tap roots, with minimum root hairs.

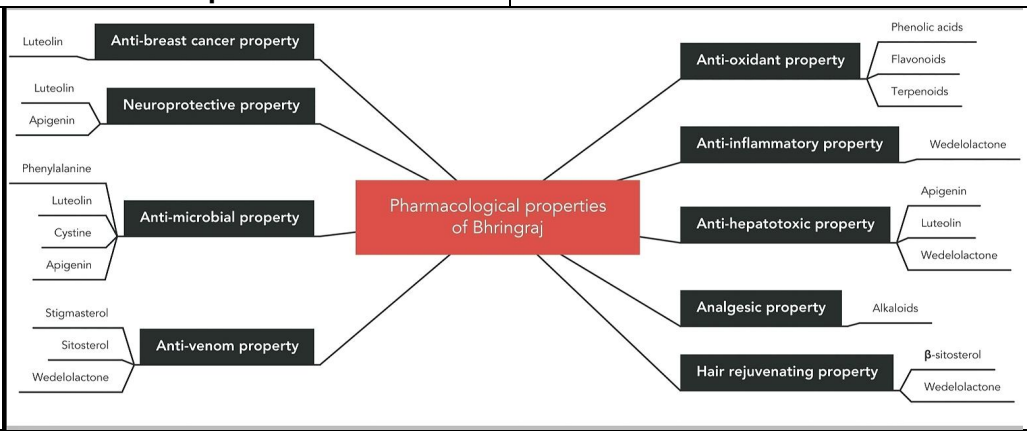

Fig 3. Phytochemicals responsible for pharmacological properties of Bhringraj

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RESEARCH ARTICLE

Ecology of Pteridophytes of Uttarakhand

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ABSTRACT

Ecological work includes the various types of the ecological adaptations. The pteridophytic flora of the study area has been classified into ecological categories *i.e.* epiphytes, lithophytes, terrestrial, aquatic and marshy, ravine, seasonal aspects and assessment of rare and endangered taxa. These ecological categories have been described briefly giving suitable examples. Besides, photographs showing various landscapes, forest types and some species of the pteridophytes have also been provided. References related to the pteridophytic flora of Uttarakhand including present study area have been given in the end.

Key words: Kumaun Himalaya, Pteridophytes, Ecology, conservation.

INTRODUCTION

Pteridophytes are an ancient evolutionary and interesting group of plants and form an important component of Himalayan flora, where the ground flora are well supplemented by epiphytic ferns. Though pteridophytes form an important part of Himalayan vegetation, yet it has remained neglected group as compared to flowering plants of Indian Himalaya. Among the earlier workers, who made significant contributions to the pteridophytic flora of Kumaun Himalaya include Clarke (1880), Beddome (1883 & 1892), Hope (1899-1904), Duthie (1906), Loyal and Verma (1960), Verma and Khullar (1980), Dhir (1980), Pangtey *et al*. (1982 &1991), Pangtey and Punetha (1987), Punetha and Kaur (1987), Pande (1990), Khullar *et al*. (1991), Khullar (1994 & 2000), Dixit and Kumar (2002), Pande and Pande (2002 & 2003), Fraser-Jenkins (2009), Joshi *et al*. (2009) and several others.

The study area (Pindari and adjacent areas) lies in northern part of Bageshwar district in Kumaun Himalaya and exhibits a fairly high degree of plant diversity and is considered as one of the most important botanically rich areas in the western Himalaya . But unfortunately no detailed study has been undertaken so far to analyse the flora and vegetation including pteridophytes of this area. Keeping in view the richness and high diversity of pteridophytic flora, the present study area was selected in order to undertake a comprehensive taxonomic as well as ecological studies.

The study area under investigation is situated in the north-western parts of the district Pithoragarh in Kumaun Himalaya and lies between 290 45′ 32′ to 290 52′ 10″ N latitudes and 800 7′ 30′ to 800 17′ 56″ E longitudes. The total area is spread over 180 km2 covering subtropical and temperate regions. The area is very rich in pteridophytic elements and grow abundantly and luxuriantly throughout the study area due to favourable climate, topography and forest vegetation *etc.* and forms the major vegetational component of the ground flora. They become more conspicuous and dominant during the rainy season growing in all possible habitats terrestrially, lithophytically and epiphytically.

MATERIAL AND METHODS

The present work is based on the detailed and careful collections of pteridophytic flora of the study area between the altitudes stretching from 1100-4000 m throughtout the year particularly during rainy season, when almost all pteridophytes have abundant and luxuriant growth and development. During the field explorations, observations on the habit, habitat, size of fronds, nature of rhizomes, scales, branching of fronds, abundance and ecology of pteridophytic flora were recorded. The method of collections, pressing, poisoning and mounting was carried following Jain and Rao (1976).

RESULTS

Ecology of Pteridophytes

Most of the ferns and fern-allies exhibit their luxuriant and vigorous growth during the rainy season particularly in the mountainous region usually above 1400 m altitude. With the commencement of monsoon about mid June, the ferns and fern-allies start growing vigourously and luxuriantly. This is due to the fact that there has been a prolonged dormancy awaiting the onset of rains. Because of high humidity in the atmosphere during July- mid September, there is a prolific growth and development of ferns and fern-allies all round on different habitats. This is particularly true for epiphytic and lithophytic taxa, which cloth the tree trunks, branches, boulders and stony walls with thickly clad felts of mosses and leafy liverworts and retain lot of moisture and thus provide coverage and protection to the growing rhizomes. In addition, there are number of hardy terrestrial species that grow throughout the year. Marked differences in altitudinal distribution can also be seen in certain taxa. On the basis of their broad hahitats, the pteridophytic flora of the study area can briefly be classified into following ecological groups.

Epiphytes

Epiphytic ferns usually prefer to grow on moist and shady tree trunks, branches, tops of trees and shrubs both in dense and partially and completely open forests. The composition and frequency of these epiphytic ferns vary considerably depending upon the altitudes, climatic conditions and nature of forests *i.e.* nature of bark and shady or open nature of canopy. Usually conifers do not harbour any epiphytes probably due to resinous bark. But at places where sufficient moisture prevails, there is a thick growth of epiphytic mosses and ferns.

Epiphyic ferns like *Pyrrosia costata* (Wall. *ex* Presl) Tag. & Iwats., *P. flocculosa* (D.Don) Ching, *P. lanceolata* (L.) Farwell, *P. porosa* (Presl) Hovenk., *Microsorum membranaceum* (D.Don) Ching, *Lepisorus nudus* (Hook.) Ching grow on the tree trunks, branches in forested and open places of several trees especially on *Mangifera indica* L., *Toona ciliata* M. Roem., *Sapium insigne* (Royle) Benth.& Hook. f., *Quercus glauca* Thunb., *Q. leucotrichophora* A. Camus, *Syzygium cumini* (L.) Skeels, *Pyrus pashia* Buch.-Ham. *ex* D.Don *etc.* All these species also grow equally as lithophytes on well moist and shaded localities between 800 and 1200 m altitudes.

While those growing above 1,200 to 2,356 m altitudes are among the majority of epiphytic ferns such as *Psilotum nudum* (L.) P. Beauv., *Huperzia hamiltonii* (Spring) Trev., *H. pulcherrima* (wall. *ex* Hook. & Grev.) Pichi serm., *Loxogramma involuta* (D.Don) Presl, *L. porcata* Price, *Arthromeris lehmanii* (Mett.) Ching, *A. wallichiana* (Spreng.) Ching, *Asplenium nidus* L., *Drynaria mollis* Bedd., *D. propinqua* (Wall. *ex* Mett.) J. Smith, *Lepisorus kashyapii* (Mehra) Mehra, *L.*

sesquepedalis (J. Smith) Fras.-Jenk., *Phymatopteris oxyloba* (Wall. *ex* Kunze) Pichi Serm., *Polypodiatrum argutum* (Wall. ex Hook.) Ching, *Polypodiodes amoena* (Wall. ex Mett.) Ching, *P. lachnopus* (Wall. *ex* Hook.) Ching, *P. microrhzoma* (Clarke *ex* Baker) Ching, *Vittaria flexuosa* Fee, *Asplenium ensiforme* Wall. *ex* Hook.& Grev., *A. laciniatum* D.Don,. It is intresting to note that among the epiphytes polypodiaceous members are most dominant

Lithophytes

This group of ferns generally grow firmly attached to rocks with their creeping rhizomes. Such species also show xerophytic characters which help them to overcome unfavourable conditions. Some of the common lithophytic ferns which grow on rock surfaces, boulders and walls are *Adiantum incisum* Forssk., *A. lunulatum* Burm., *Cheilanthes dalhousiae* Hook., *C. doniana* Fras.-Jenk. & Khullar, *C. dubia* Hope, *C. rufa* D.Don, *Botrychium lanuginosum* Wall. *ex* Hook. & Grev., *Pteris pseudoquadriaurita* Khullar, *Trichomanes insigne*, *Hymenophyllum exertum* (Wall. *ex* Hook.) Bedd., *Hypodematium crenulatum* (Forssk.) Kuhn, *Asplenium dalhousiae* Hook., *A. unilaterale* Lam., *Sphenomeris chinensis* (L.) Maxon, *Polystichum lentum* (D.Don) Moore, *P. mehrae* Fras.-Jenk. & Khullar, *P. nepalensis* (Spreng.) C.Chr., *P. thomsonii* (Hook.f.)Bedd., *Tectaria macrodonta* (J. Smith) C.Chr., *T. polymopha* (Wall. *ex* Hook.) Copel.,

Among the fern-allies, *Selaginella* species usually prefer to grow on rocks and boulders such as *S. chrysocaulos* (Hook. & Grev.) Spring, *S. involvens* (Sw.) Spring, *S. pallida* (Hook. & Grev.) Spring, *S. pallidissima* Spring and *S. subdiaphana* (Wall. *ex* Hook. & Grev.) Spring. Among these species, *S. involves* also grows as foot epiphyte. All these specis are common throughout the study area and they grow forming mats on rocks and walls in the forest, forest margin, waysides and roadsides. They are particularly conspicuous during rainy and autumn seasons.

Terrestrial Ferns

A good number of ferns of the study area consists of terrestrial ferns. They generally grow in open places, humus rich forest floor, forest margin, waysides and roadsides forming a conspicuous vegetation. Some common terrestrial ferns are: *Dicranopteris linearis* (Burm. *fil.*) Underw., *Pteris biaurita* L., *P. cretica* L., *P. excelsa* Gaud., *P. pseudoquadriaurita* Khullar, *P. subquinata* Wall. *ex* Agardh, *P. wallichiana* Agardh,, *P. vittata* L., *Onychium crypogrammoides* Christ, *O. japonicum* (Thunb.) Ktze., *O. siliculosum* (Desv.) C.Chr., *Pteridium aquilinum* (L.) Kuhn var. *wightianum* Tryon, *Adiantum edgeworthii* Hook., *A. venustum* D.Don. Several lithophytic species may also tend to grow terrestrially on forest floor, forest margin, open places, roadsides and waysides and the common ones are the members of *Athyrium*, *Dryopteris*, *Polystichum*, *Pteris*, *Diplazium etc.* One species of tree fern (*Cyathea spinulosa* Wall. *ex* Hook.) grows in the forests all along the perennial streams and sometimes in open places in moist and shady situations in the north aspects.

Aquatic and Marshy Ferns

Aquatic and marshy ferns grow along the banks of perennial streams and water falls *etc.* and form a quite conspicuous and rich vegetation. They include *Angiopteris evecta* (Forst.) Hoffm., *Diplazium bellum* (Clarke) Bir, *D. esculentum* (Retz.) Sw., D. *maximum* (D.Don) C.Chr., *D. spectabile* (Wall. *ex* Mett.) Ching, *D. subsinuatum* (Wall. ex Hook. & Grev.) Tag., *Tectaria polymorpha* (Wall. *ex* Hook.) Copel., *Deparia japonica* (Thunb *ex* Murray) Kato, *Dennstaedtia scabra* Wall. *ex* Moore *etc.* Besides, *Equisetum diffusum* D.Don and *E. ramossisimum* Desf. grow profusely in marshy and wet places covering considerable areas and form big colonies. However, no true aquatic ferns have been observed in the study area.

Ravine Ferns

Ravine ferns require more humidity and moisture than the ferns growing in open places. Therefore they usually prefer to grow in the ravines near perennial water courses, which provide them suitable substratum for their growth and development throughout the year. Ferns which grow commonly in the ravines are: *Diplazium bellum* (Clarke)Bir, *D. maximum* (D.Don) C.Chr., *D. polypodiodes* Blume, *Glaphyropteridopsis erubescens* (Wall. *ex* Hook.) Ching, *Cyclogramma auriculata* (J. Smith) Ching, *Pseudocyclosorus canus* (Baker) Holtt. & Grimes, *P. tylodes* (Kunze) Ching, *Pseudophegopteris pyrrhorhachis* (Kunze) Ching, *Pronephrium nudatum*, *Coniogramme pubescens* Hieron., *Woodwardia unigemmata* (Makino) Nakai, *Cyrtomium caryotideum* (Wall. *ex* Hook. & Grev.) Presl, *Polystichum nepalense* (Spreng.) Presl and many others.

Thicket Forming Species

There are some tough and hardy species which grow on exposed rocky and gravelly slopes. They are usually exposed to a wide range of variations of climatic conditions such as high and low wind velocities, marked temperature fluctuations and varied amount of moisture. Some examples are: *Pteridium revolutum* (Blume)Nakai*aquilinum* (L.) Kuhn var. *wightianum* Tryon forms huge patches in recently exposed rideges, on forest floor, forest margin, grasslands, on highly exposed, dry and open situations. Rhizomes are deeply buried under the soil and creep long distances with wide branching. The fronds form tangled mass and thus do not allow anything to grow.

Seasonal Aspects

The varying nature of climatic conditions also influence the growth and development of ferns and fern-allies. The ferns of the study area like other parts of mountainous regions also exhibits a marked seasonality among the species. It has been observed that most of the species flourish during rainy season between mid June to mid September. With the advent of first showers of rain and hearly heat of summer (April to June), nearly all the species put forth new fronds which grow vigorously and become fertile during August to September. These species suddenly cover different habitats till September.

With the commencement of winter season (November to March), all the epiphytic and lithophytic species undergo dormancy after shedding their fronds due to prevailing unfavourable climatic conditions. During this period, only shrivelled up or enrolled dry, yellowish fronds of certain epiphytic and lithophytic species like *Loxogramme involuta* (D.Don) Presl, *Asplenium indicum* Sledge, *Pyrrosia costata* (Wall. *ex* Presl) Tag. & Iwats., *P. flocculosa* (D.Don) Ching, *P. lanceolata* (L.) Farwell, *P. porosa* (Presl) Hovenk., *Vittaria flexuosa* Fee, *Lepisorus nudus* (Hook.) Ching *etc*. are observed.

The aerial parts die off completely or partially giving sudden disappearance of these species. But the undergrown and creeping rhizomes covered by leafy bases, scales and hairs help them to overcome unfavourable conditions during winter season and become active with the beginning of spring season. However, some species of hardy ferns like *Polystichum discretum* (D.Don) J.Smith, *P. squarrosum* (D.Don) Fee, *Hypolepis punctata* (Thunb.) Mett., *Adiantum capillus-veneris* L., *A. venustum* D.Don, *Tectaria macrodonta* (J.Smith) C.Chr., *Pteris excelsa* Gaud., *P. pseudoquadriaurita* Khullar, *P. vittata* L., *P. wallichiana* Agardh, *Woodwardia unigemmata* (Makino) Nakai *etc*. remain green throughout the year, although their new fronds are put forward only after the spring. During winter months, the aerial parts of almost all terrestrial species are killed completely or partially by snow and frost above 1,500 m altitude, but species growing on walls and other protected situations remain green throughout the winter season. With the advent of spring together with summer heat and pre-monsoon showers, the area resumes its dense growth of vegetation.

REFERENCES

1. Beddome, R. H. (1883). *A Handbook to the Ferns of British India, Ceylon and Malay Peninsula*. Calcutta.
2. Beddome, R. H. (1892). *A Handbook to the Ferns of British India, Ceylon and Malay Peninsula with Supplement*. Calcutta.
3. Clarke, C. B. (1880). A review of ferns of northern India. *Trans. Linn. Soc. Lond. II. (Bot.)* 1: 425-611.
4. Dhir, K. K (1980). Ferns of North Western Himalayas. *Bibliotheca Pteridologia* 1: 1-158.
5. Dixit, R.D. and R., Kumar (2002). *Pteridophytes of Uttarakhand (A Check List).* Dehradun.
6. Duthie, J. F. (1906). *Catalogue of the Plants of Kumaon and of the Adjacent Portions of Garhwal and Tibet, based on the collections made by Strachey and Winterbottom during the years 1846 to 1849 and on the catalogue originally prepared in 1852 by Sir Richard Strachey*. London.
7. Fraser-Jenkins, C.R.(2009*).Taxonomic Revision of Three Hundred Indian Subcontinental Pteridophtes with a Revised Census-List.* Dehra Dun.
8. Hope, C. W. (1899-1904). The ferns of Nort-Western India. *J. Bombay nat. Hist. Soc.* 12: 315-325, 527-538, 62-633 (1899); 13: 25-36, 236-251 (1900); 443-461, 657-671 (1901); 14: 119-127, 252-266, 458-480 (1902); 720-749 (1903); 15: 67-111, 415-429 (1904).
9. Jain, S.K. and R.R.Rao (1976). *A Handbook of Field and Herbarium Methods*. New Delhi.

10. Kaur, S. and N. Punetha (1984). A new variety of *Dicranopteris linearis* (Burm. f.)
11. Khullar, S. P. (1994). *An Illustrated Fern Flora of West Himalaya*. Vol. I. (Botrychiaceae to Aspleniaceae). Dehradun.
12. Khullar, S. P. (2000). *An Illustrated Fern Flora of West Himalaya*. Vol. II (Onocleaceae to Salviniaceae). Dehradun.
13. Khullar, S. P., Anurita Sharma and S. S. Samant (1991). A new species of the genus *Elaphoglossum* from the Kumaun Himalaya. *Indian Fern J.* 8: 1-4.
14. Pande, H.C. and P.C.Pande (2003). *An Illustrated Fern Flora of the Kumaon Himalaya*. Vo. I & II. Dehradun.
15. Pande, P.C. and H.C. Pande (2002). An updated catalogue of ferns and fern allies of Garhwal Himalaya. *Indian J. Forestry* 25:486-498.
16. Pangtey, Y. P. S. and N. Punetha (1987). Pteridophytic flora of Kumaun Himalaya : An up dated list.In : *Western Himalaya (Environment, Problem & Development), ed*. Y. P. S. Pangtey & S. C. Joshi, Naini Tal. 1: 389-412.
17. Punetha, N. and S. Kaur (1987). Pteridophytic flora of Pithoragarh district (North-Western Himalaya). *J. Econ. Tax. Bot*. 9: 269-286.
18. Rao, T. A. (1959). Report on a botanical tour to Milam glacier. *Bull. Bot. Surv. India* 1: 97

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RESEARCH ARTICLE

Phytochemical Screening, Quantitative Analysis of Total Phenols and Total Flavonoids and Antioxidant Activity of *Berrya cordifolia* (Willd.) Burret Leaf Extract.

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ABSTRACT

Berrya cordifolia (Willd.) Burret belongs to family Malvaceae is comparatively understudied. The aim of this present study was to investigate the presence of phytochemicals, total phenolic content, total flavonoid content and antioxidant activity of the selected plant leaf extract. Maceration method was used for the extraction process. The leaf of *B. cordifolia* was extracted using solvents like Methanol and Di-ethyl ether. The phytochemical constituents were determining using standard qualitative methods. Total phenolic content of the methanolic leaf extract was determined by Folin-Ciocalteu reagent method whereas total flavonoid content of the methanolic leaf extract was determined by the Aluminium Chloride method. Antioxidant activity of the extract was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Preliminary phytochemical screening of methanolic and Di-ethyl ether extract of *B. cordifolia* indicates the presence of alkaloids, carbohydrates, glycosides, proteins, tannins, phenols, flavonoids, terpenoids, cardiac glycosides, and steroids. The Total phenolic content, expressed as mg of Gallic acid equivalent (GAE) per gram of extract was found to be 316.666 ± 6.009 mgGAE/g and Total flavonoid content, expressed as mg of Quercetin equivalent (QE) per gram was found to be 163.333 ± 1.666 mg QE/g. Free radical scavenging activity (DPPH) which measured by IC_{50} value, for the leaf extract IC_{50} value is 106.882 ± 0.2977 µg / ml and for ascorbic acid standard IC_{50} value found to be134.8814 ± 0.3006 µg / ml. These studies provided information for standardization and correct identification of this plant material. The methanol leaf extract of *B. cordifolia* showed the highest yield. The diverse array of phytochemicals present in the plant suggests its significant therapeutic potential which may be explored in drug manufacturing industry as well as in traditional medicine. The study

suggested that the methanolic leaf extract have more potential in scavenging the free radicals as compared to standard ascorbic acid.

Keywords: *Berrya cordifolia*, Phytochemicals, Total phenols, Total Flavonoids, Antioxidant activity

INTRODUCTION

The ancient beliefs show the relation of human beings and plants [1]. The medicinal use of plants has been recognised in practically every ancient civilisation. Around 80% of the world's population is still reliant on conventional medication for their health treatment [2]. Plants are indispensible source of medicine since time immemorial. Studies on natural products are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential therapeutic agents [3]. The medicinal properties of plants are due to some chemical constituent that produce certain pharmacological action on the humans [4]. In developed countries, 25% of the medicinal drugs are based on plants and their derivatives [3]. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs [3]. Since plants are the natural storehouse of drugs, they are derived directly or indirectly from these botanicals and are known to have different medicinal properties [5].

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefit for humans as medicinal ingredients [6]. They protect plants from disease and damage, and also contribute to the plant's colour aroma and flavor [7]. In general the plant chemicals that protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals [8]. Phytochemicals are primary and secondary metabolites, which are naturally occurring in leaves, vegetables and roots that have defence mechanism and protect from various diseases [9]. Primary metabolites are protein, carbohydrates, chlorophyll, lipids, common sugar which is synthesized during photosynthesis, and these organic compounds are essential for plant life and growth and development [9]. Secondary metabolites are flavonoids, alkaloids, saponins, phenols, and tannins, which are synthesized by the plant during development [10]. Natural products extracted from plants such as terpenoids, alkaloids, and flavonoids have received a lot of attention in recent years because of their various pharmacological properties, including antimicrobial, antioxidant, and anticancer activities [11].

Family Malvaceae consist of approximately 244 genera and 4225 species [12]. It is one of the largest families of angiosperms. Many plants of this family have been used in traditional system of medicine from ancient time; still there are certain medicinal plants whose medicinal properties have not been explored properly. Moreover, several plant genera are extensively explored for phytochemicals with regard to their ethanomedicinal properties. The genus *Berrya* belongs to the Malvaceae family, which is part of the major group of angiosperms (flowering plants)[13]. The genus *Berrya* has six accepted species names. *Berrya cordifolia* (Willd.) Burret (Figure.1) is one of these genera species, also known as "Trincomalee wood" or "Halmila tree," is a tropical Asian native tree that has been brought to Africa. It is a genus of evergreen trees from Southeast Asia and the Pacific region with fibrous bark.

It was kept under Tiliaceae family but according to APG system Ⅲ it is belong to Malvaceae family [13,14]. It is distributed in Kerala, Maharashtra, Tamil Nadu and Andaman Island in India. The plants are valuable for their timber. It was also found in the forest of Christmas Island [13]. The flowers are showy, with large clusters of green flowers. Trees from *Berrya* are propagated from seed and grown in warm temperature or tropical climates. Hairs on the seeds cause skin irritation. It is widely used for Timber, and its bark is used for fibers. Large wooden vats, called wash backs made from the wood of teak or *Berrya cordifolia* the natural fermentation process is allowed to continue in the wash backs. Therefore, this experiment is aimed to investigate the phytochemical screening, including total flavonoid content, total phenolic content and antioxidant activities of *B.cordifolia* leaf extract.

MATERIAL AND METHODS

Collection and Identification

Berrya cordifolia (Willd.)Burret (Trincomalee wood) tree leaves were collected from St. Xavier's college campus, Ahmedabad, Gujarat, India, in January 2021 (latitude 23°01'55.1'' N and longitude 72°33'04.1'' E). The plant identification and pre-treatment was carried out immediately.

Plant material

The fresh samples (leaves) were collected and washed twice using distilled water to remove soil particles and other dust. The leaves were air dried at the room temperature for 15 days. The dried leaves sample were ground to a fine powder with the help of mixer grinder. The leaves powder was stored in airtight container for further analysis.

Preparation of plant extract

The maceration method was used to prepare plant extract. A 10gm leaves powder was soaked into 100ml organic solvent like Methanol (polar) and Di-ethyl ether (non-polar), separately for 24hrs in an orbital shaker at normal temperature at constant stirring rate at 112 rpm. The extract was filter through the Whatman No.1 filter paper, and extra solvent was evaporated then stored at 4° C for further analysis. Finally the yield value of crude extract was calculated by given standard formula.

$$\% \text{ Yield} = \frac{\text{Weight of dry extract}}{\text{Weight of plant powder}} \times 100$$

Preliminary Phytochemical Analysis

Phytochemical screening was performed to identify potent phytochemicals in the Methanol and Di-ethyl ether extracts of plant leaves. In the present study the phytochemicals were detected by colour tests. The qualitative phytochemical analysis of crude extract was done using the following standard method [15, 16].

Test for alkaloids

Mayer's test: Take 2 ml of each extract then add 2 ml Mayer's reagent to the side of test tube. The appearance of white creamy precipitate indicates the presence of alkaloids.

Wagner's test: In the test tube take 2 ml of extract and add few drops of Wagner's reagent to the side of the test tube. The appearance of reddish brown precipitate indicates the presence of alkaloids.

Hager's test: About 2ml of extract was added in the test tube then 1-2 drops of Hager's reagent were added in it. The appearance of yellow precipitate indicates the presence of alkaloids.

Dragendroff's test: Take 2ml of each extract then add 2 ml Dragendroff's reagent. The appearance of orange precipitate indicates the presence of alkaloids.

Test for carbohydrates

Molish test: Take 2 ml of extract and add few drops of Molish reagent to the side of the test tube. Violet coloured ring indicates the presence of carbohydrates.

Fehling's test: Equal volume of Fehling A and B reagent were mixed together and add in 2 ml of extract and gently boiled over water bath. A red precipitate indicates the presence of carbohydrates.

Barford test: In test tube 2 ml of extract mixed with 2 ml barford reagent boil in the water bath for 2 minute. Appearance of red colour precipitate indicates the presence of carbohydrates.

Benedict test: Take 2ml of extract and 2ml Benedict reagent was boiled over water bath for 2 minute. The green coloured precipitate indicates the presence of carbohydrates.

Test for glycosides

Borntrager's test: Take 2 ml of extract mixed with 3 ml chloroform and 2 ml acetic acid. Then added few drops of concentrated H_2SO_4 then cooled on ice. Violet- blue- green colour indicates the presence of glycosides.

Borntrager's test: Take 2 ml of extract mixed with 3 ml of chloroform and shaken well, add 10% ammonia solution. Pink coloured precipitate indicates the presence of glycosides.
Keller-Killani test: In the test tube add 2 ml of extract then add 1 ml glacial acetic acid containing 2% of $Fecl_3$ followed by 1 ml concentrated H_2SO_4. Two layers are formed upper red brown layer and lower blue green layer indicates the presence of carbohydrates.

Test for proteins

Millon's test: Each 2 ml of extract mixed with few drops of Millon's reagent. A white coloured precipitate indicates the presence of proteins.
Biuret test: Take 2 ml of extract mixed with 1 drop of 2% copper sulfate solution ($CuSO_4$) and add 2 ml of 95% ethanol and 1 KOH pellets. Pink coloured solution (in ethanolic layer) indicates the presence of proteins.

Test for phenols

Ferric chloride test: In test tube 2 ml of extract is treated with few drops of 5% $FeCl_3$ solution. Formation of dark green colour indicates the presence of phenols.

Test for Tannins

Lead acetate test: In test tube containing 2 ml of extract and 1 ml of lead acetate was added. White coloured precipitate indicates the presence of tannins.
Ferric chloride test: Take 2 ml of extract then add few drops of 5% $FeCl_3$ solution in the test tube. Formation of dark green colour indicates the presence of tannins.
Folin-ciocalteu test: Take 2 ml of extract then add 1 ml Folin-cioculteu reagent was added in the test tube. Bluish green colour indicates the presence of tannins.

Test for Flavonoids

Alkaline reagent test: Take 2 ml of extract mixed with 2 ml of 2% NaOH solution and add few drops of dilute HCl. An intense yellow colour becomes colourless on addition of diluted acid indicates presence of flavonoids.
Lead acetate test: To 2 ml of each extract and few drops of 10% Lead acetate. The appearance of yellow colour precipitate indicates the presence of flavonoids.

Test for Saponins

Forth test: Take 2 ml of extract taken in a test tube, 20 ml of distilled water was added and mixture was shaken vigorously and kept for 3 min. A honey comb like froth was formed and it showed the presence of saponins.

Test for Terpenoids

Copper acetate test: Take 2 ml of extract and add few drops of lead acetate solution in the test tube. Emerald green colour indicates the presence of terpenoids.
Salkowski's test: Two ml of each extract was mixed in 2 ml of chloroform, add concentrated H_2SO_4 (1ml). Golden yellow colour ring indicates the presence of terpenoids.

Test for cardiac glycosides

Legal test: Two ml of each extract was mixed in 1 ml pyridine, and add 20% of sodium nitroprusside and few drops of sodium hydroxide. Formation of pink or red colour precipitate indicates presence of cardiac glycosides.

Test for steroids

Salkowski's test: Chloroform 1 ml was added to 2 ml of extract and shake it well and add concentration H_2SO_4 side by side. Red colour indicates the presence of steroids.

Liebermann-Burchard's test: Take 2 ml of each extract. To these extracts 2-3 ml of acetic anhydride solution was added and then 2 ml of H_2SO_4acid was added along the sides of the test tube. The appearance of violet or green colour indicates the presence of steroids.

Total Phenolic Content

Chemicals: Folin-Ciocalteu reagent, Gallic acid (Standard), 20% Sodium Carbonate, Distilled water, Methanol, Plant extract

Procedure for determination of total phenolic content: The total phenolic content of methanolic leaf extract was determined by Folin-Ciocalteu method. Gallic acid was used as standard and the total content of phenol in extract was expressed in mg/g Gallic Acid Equivalents (GAE) [17]. The Gallic acid was prepared in methanol at a concentration of 1mg/5ml. The plant extract was also prepared in methanol at a concentration of 1mg/5ml and the reaction mixture was prepared by mixing 1ml of extract solution with 1.5ml Folin-Ciocalteu reagent dissolved in 10ml water. Add 4 ml of 20% Sodium Carbonate to make total volume 25 ml with distilled water in each test tube. The test tubes covered with Aluminum foil and allowed to incubate for 30 minute at room temperature. The absorbance was measured at λ 765 nm using spectra against blank. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the solution of Gallic acid (Standard). The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total phenolic content can be determined.

The formula to find Gallic acid equivalent of plant sample is as followed:

GAE= C ×V/ M

Where, C = Concentration of gallic acid obtained from the calibration curve in mg/ml

V = Volume of the extract solution in ml

M = Weight of the extract in g

Total Flavonoid Content

Chemicals: 10% Aluminum Chloride ($AlCl_3$) reagent, 1 M Potassium Acetate (CH_3COOK), Quercetin (Standard), Distilled water, Methanol, Plant extract

Procedure for determination of total flavonoid content: The total Flavonoid content of methanolic leaf extract was determined by Aluminum chloride colorimetric method. Quercetin was used as standard and the total content of flavonoid compound in extract was expressed in mg/g Quercetin equivalent [17]. The Quercetin and plant extract were prepared in methanol at a concentration of 1mg/5ml and the reaction mixture was prepared by mixing 1 ml of extract solution, 100µL 10% $AlCl_3$ and 100µL 1M Potassium acetate reagent dissolved in 4.8 ml water in each test tubes. The test tubes covered with Aluminum foil and allowed to incubate for 30 minute at room temperature. The absorbance was read at λ 415 nm using spectra against blank. The sample was prepared in triplicates for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the solution Quercetin (standard).

The formula to find Quercetin equivalent of plant sample is as followed:

QE = C × V/M

Where, C = Concentration of gallic acid obtained from the calibration curve in mg/ml

V = Volume of the extract solution in ml

M = Weight of the extract in g

Antioxidant activity

The antioxidant activity of methanolic extract of *B. cordifolia* leaves determined using DPPH free radical scavenging assay (DPPH of Sigma-Aldrich Co., Germany). To determine antioxidant activity 2,2-diphenyl-1-picryl-hydrezyl

(DPPH) was used as free radical. DPPH was prepared by dissolving the 4 mg (0.004%) of DPPH in 100 mL methanol. 2 ml of prepared DPPH was applied to different concentrations of sample (40, 80, 120, 180, 200 µg/ml) and incubated for 20-30 minutes in dark at room temperature. The absorbance was measured in triplicates in a spectrophotometer at 517 nm for each concentration. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, thus a low value corresponds to a good scavenging ability. Ascorbic acid solution of same concentration can be used as standard for the free radical scavenging assay. The results were expressed as IC_{50} value that is the amount of antioxidant necessary to decrease by 50% of the initial DPPH radical concentration [18].

The potential ability to scavenging DPPH free radical was estimated by the below equation:

% inhibition ratio = $[(A_{Control}–A_{sample})/A_{Control}] \times 100$

Absorbance of control ($A_{Control}$) = absorbance of solution that contain all reagents except sample

Absorbance of sample (A_{sample}) =absorbance of sample or standard

Statistical Analysis

Qualitative and graphical data was analysed using Microsoft Excel. All tests were carried out in triplicates and the results of each series of experiments were expressed as mean values ± standard deviation.

RESULTS

Yield form extracts:

After collecting and drying fresh *Berrya cordifolia* leaves, approximately 49 g powder was obtained. Yields of different extracts are shown in table1. The yield in *B. cordifolia* leaf was maximum in methanol extract (1.194 g) and least in Di-ethyl ether (0.145 g). Nature of the crude extracts is shown in table 2.

Phytochemical screening

The result of qualitative phytochemical screening of leaf of *B. cordifolia* revealed the presence of alkaloids, carbohydrates, glycosides, proteins, tannins, phenols, flavonoids, terpenoids, cardiac glycosides, steroids as mention in table 3.

Total phenolic content

In this study total phenolic content of methanolic extract of *B. cordifolia* leaves was estimated using linear equations derived from gallic acid standard curve. Calibration curve calculated using linear equation obtained from Gallic acid ($y = 0.004x - 0.097$; $R^2 = 0.989$) showed maximum absorbances at 765 nm wavelength. The Folin-Ciocalteu method was used to determine the total phenol content of leaf extracts, which was reported as gallic acid equivalent (Figure 2). The leaf extract of *B. cordifolia* contained significant amount of total phenol content is 316.666 ± 6.009 mg GAE/g.

Total Flavonoid content

In this study Total flavonoid content of methanolic leaf extract of *B. cordifolia* was estimated using the standard curve plotted for Quercetin ($y = 0.012x + 0.118$; $R^2 = 0.991$) which showed maximum absorbance at 415 nm. Where y denotes absorbance at 415 nm wavelength and x denotes total content of flavonoid content. Leaf extract contains 163.333 ± 1.666mgQE/g of total flavonoid content (Figure 3).

Antioxidant activity

DPPH assay was used to assess the antioxidant activity of methanolic leaves extract of *Berrya cordifolia* at various concentrations. In antioxidant activity, ascorbic acid is considered as a standard equivalency. The DPPH assay can be used to determine the presence of antioxidant potential in extract. In the presence of antioxidant potential, DPPH changes colour from purple to mustard yellow. The IC_{50} value was determined by plotting inhibition (%) against concentration value and was expressed in milligrams per millilitre of extract. The IC_{50} value of *B. cordifolia* leaves

extract found to be 106.882±0.2977µg/ml and the standard ascorbic acid IC_{50} value is 134.8814 ± 0.3006µg/ml. The results shows that the *B. cordifolia* leaves extract had significantly higher antioxidant activity.

DISCUSSION

Berrya cordifolia (Willd.)Burret is mainly used as timber tree. The main purpose of this research was to investigate and elucidate the medicinal values of *B. cordifolia.* This is the first study that we are reporting since there is less prior literature on the phytochemical characterization of the leaves of this plant. Consequently, *Berrya cordifolia* was selected and standardized on their phytochemical study, qualitative estimation, TPC and TFC analysis, antioxidant activity for the examination. Present study of *Berrya cordifolia* revealed the presence of alkaloids, carbohydrates, glycosides, proteins, tannins, phenols, flavonoids, terpenoids, cardiac glycosides, steroids in methanolic and Di-ethyl ether leaves extract, the proteins are absent in Di-ethyl ether extract. The other member from the same family Malvaceae shows the phytochemical analysis. According to Dhalwal *et al*. [19], reported in *Sida cordifolia* phytochemical analysis confirmed the presence of alkaloids and flavonoids. *Hibiscus sabdariffa* revealed the presence of phytochemicals like tannins, saponins, glycosides, phenols and flavonoids [20]. Deora & Bano [12], investigate the primary qualitative phytochemical screening of *Abutilon pannosum* revealed the presence of carbohydrates, amino acids, alkaloids, phenols, flavonoids, phytosterols, tannins, terpenoids and glycosides. In the *Sida acuta* presence of phytochemicals like alkaloids, steroids, flavonoids, tannins, and glycosides presence in chloroform extract [21]. According to Shukla *et al.* [22], the hydro-methanolic root extract exhibit the presence of tannins, flavonoids, alkaloids, phenols, saponins, glycosides, and proteins in *Sterculia urens*. In aqueous leaf extract of *Grewia hirsuta* the presence of alkaloids, phenols, tannin, glycosides, steroids and terpenoids [23]. According to Sadat *et al.* [24] presence of carbohydrate, glycosides, proteins, steroids, alkaloids, phenols, flavonoids, and saponin are present in methanolic immature leaf of *Corchorus olitorius*.

B. cordifolia displayed notable concentration of TPC which is supported by various studies in the same family. Shukla *et al.* [22], found a maximum of 705± 0.40 mg GAE/g in Sterculia urens, however our findings for TPC are lower. Subramanya *et al.* [25], evaluated different *Sida* species that *Sida cordifolia* has 1.26± 0.06 mg QE/g total flavonoid content and *Sida acuta* has 0.84± 0.04 mg QE/g total flavonoid content. According to Ali *et al.* [18], total phenol content of *Urena lobata* is 211.95 µg / ml and 230.40 µg / ml is total flavonoid content present. The selected species *Berrya cordifolia* possess higher flavonoid content (163.333 ± 1.666 mg QE/g) than *Sida cordifolia*. Phenolic compounds act as antioxidant because of their redox properties. Corresponding outcomes were reported by Shukla *et al*. [22]; the *Sterculi aurens* root crude methanolic extract has an IC50 value about 27.055 µg / ml. In the *Sida acuta* the DPPH scavenging activity of different extract decreased in the order to root, stem, leaf, and whole plant were it found to be 76.62%, 63.87%, 58%, and 29% respectively [19]. According to Subramanya *et al.* [25], *Sida retsa* has 17.42± 0.87 scavenging activity, and *Sida cordifolia* has 51.31± 2.57 radical scavenging activity. The IC50 value in *Gossypium herbaceum* is 44.69µg/ml [26]. According to Ali *et al.* [18], the IC50 of the standard and methanol extract of *Urena lobata* were 150 µg/ml, and 180 µg/ml respectively, here the plant extract possess potential antiradical activity. *Chorchorus olitorius* immature leaf methanolic extract have 27.563±2.813 % at 200 µg/ml DPPH free radical scavenging activity [24]. In *Hibiscus asper* antioxidant activity of calyx is 53.33 ± 0.25% and the polyphenols of calyx is 59.27 ± 0.31 GAE/g [27]. In leaf of *Hibiscus asper* antioxidant activity is 36.33 ± 0.45% and polyphenols of the leaf is 72.50 ± 0.20 GAE/g [27]. The IC50 value of methanolic leaf extract was determined to be 106.882 ± 0.2977µg/ml in the current study of *Berrya cordifolia*.

The principle of this study is to identify the phytoconstituents, quantify the total phenols and total flavonoids and also assess the antioxidant activity of *Berrya cordifolia*. The phytochemical screening showed that *B. Cordifolia* leaf extract possesses a wide range of phytochemicals including Alkaloids, flavonoids, carbohydrates, glycosides, proteins, tannins, phenols, terpenoids, cardiac glycosides and steroids. Among the all compound, phenols and flavonoids are important constituent for antioxidant potentiality of the plant. The methanolic leaf extract of *B.*

cordifolia shows the significant amount of phenols and flavonoid content and thus shows effective antioxidant activity. Thus, the present work suggests that *B. cordifolia* has potential pharmaceutics and medicinal efficacy for the pharmaceutical industries to use as a source for developing new therapeutics drug. This is the main reason to selecting this plant as it is still quite unexplored and thus requires more research for its hidden properties to cure various diseases. However, it has not received the deserved attention and will be investigated further to their various pharmacological aspects.

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REFERENCES

1. Pandey, Deepti, and Vimal Chandra Pandey. "Sacred plants from ancient to modern era: Traditional worshipping towards plants conservation." *Tropical Plant* Research (2016); 3.1: 136-141
2. Doss, A. Preliminary phytochemical screening of some Indian medicinal plants. *Ancient science of life*,(2009); 29(2), 12.
3. Nidavani, R. B., & Mahalakshmi, A. M. Teak (Tectonagrandis Linn.): a renowned timber plant with potential medicinal values. *International Journal of Pharmacy and Pharmaceutical Science* (2014); *6*(1), 48-54.
4. Karole, S., Gautam, G., & Gupta, S. Physicochemical, Qualitative and Quantitative Phytochemical Analysis of the Leaf and Bark of Bombaxcieba L (Red Silk Cotton Tree). *Journal of Drug Delivery and Therapeutics* (2018); *8*(6-s), 105-110.
5. Gulnaz, A. R., Kapfo, W., &Chauhan, J. B. In vitro anthelminathic and anti-inflammatory activity of ethanolic extract of Randiauliginosa DC Leaf. *Int. J. Curr. Microbiol. App. Sci*(2014);3(9), 793-799.
6. Hasler, C. M., & Blumberg, J. B. Phytochemicals: biochemistry and physiology Introduction. *The Journal of nutrition* (1999)*;* 129(2), 756S-757S.
7. Gibson, E. L., Wardle, J., & Watts, C. J. Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. *Appetite*, (1998); *31*(2), 205-228.
8. Koche, D. E. P.A.K., Shirasat, R. U. P.A. L. I., & Kawale, M. A. H. E. S. H. An over view of major classes of phytochemicals: their types and role in disease prevention. *Hislopia Journal*(2016); *9*, 1-11.
9. Wadood, A., Ghuafran, M., Jamal, S. B., Naeem, M., Khan, A., & Ghaffar, R. Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochem Anal Biochemist*(2013); *2*(4), 1-4.
10. LingaRao, M., & Savithramma, N. Quantification of primary and secondary metabolites of Svensoniahyhyderobadensis-A rare medicinal plant. *Int J Phar pharm Sci,*(2012); *4*, 519-21.
11. Takeoka, G. R., & Dao, L. T. Antioxidant constituents of almonds [Prunusdulcis (Mill.) DA Webb] hulls. *Journal of Agricultural and Food Chemistry,*(2003); *51*92), 496-501.
12. Deora, G. S., &Bano, I. Preliminary phytochemical screening and GC-MS analysis of methanolic leaf extract of Abutilon pannosum (Forst. F.) Schlect. From Indian Thardesert. *J Pharmacogn Phytochem* (2019);*8*(1), 894-99.
13. Sankara Rao, K., Raja K Swamy, Deepak Kumar, Arun Singh R. And K. Gopalakrishna Bhat. Flora of Peninsular India (2019).
14. Lrano, A. A. P., &BuotJr, I. E. Leaf architecture of selected species of Malvaceaesensu APG and its taxonomic significance. *Philippine Journal of Systematic Biology,* (2010); 4, 21-54.
15. Gowri, S. S., & Vasntha, K. Phytochemical screening and antibacterial activity of *Syzgium cummini* (L.)(Myrtaceae) leaves extracts. *Int J Pharm Tech Res,* (2010); 2 (2), 1569-1573.
16. Das, A., Bharath, M., Jeevanatham, M., Kumar, S. M., & Bindhu, R. P. Phytochemical Screening and antimicrobial activity of *Syzgium cumini* (Jamun) seed Extract. *Research Journal of Pharmacy and Technology,* (2018); *11* (9), 4096-4100.
17. Bora, J., Syiem, D., & Bhan, S. Quantitative analysis of total phenolic, flavonoid. *J. Pharmacognosyphytother,* (2019);8,906-911.

18. Ali, M. S., Faruq, K. O., & Rahman, M. A. A Antioxidant and cytotoxic activities of methanol extract of *Urena lobata* (L) Leaves. *The Pharma Innovation,* (2013); *2*(2).
19. Dhalwal, K., Deshpande, Y. S., Purohit, A. P., & Kadam, S. S. Evaluation of the Antioxidant Activity of *Sida cordifolia*. *Pharmaceutical biology,*(2005); *43*(9), 754-761.
20. Nkumah, O. C. Phytochemical analysis and medicinal uses of Hibiscus sabdariffa. *International journal of Herbal medicine*, (2015); *2*(6), 16-19.
21. Muneeswari, P., Deepika, S., Perumal, P. C., Gopalkrishnan, V. K., & Poornima, K. Phytochemical screening and free radical scavenging activity of chloroform extract of SidaacutaBurm. F. *International Journal of Pharmacognosy and phytochemical Research,*(2016); *8*(4), 663-667.
22. Shukla, A, Desai, K., & Modi, N. In vitro antioxidant and antimicrobial potential of *Sterculi aurens* Roxb. root extract and its bioactive Phytoconstituents evaluation. *Future Journal of Pharmaceutical Sciences,*(2020); *6*(1), 1-11.
23. Hutke, V. D., & Naswale, M. Screening of various extracts of Grewiahirsuta leaves and fruits for secondary metabolites. *Journal of Medicinal Plants,* (2017); *5*(1), 323-325.
24. Sadat, A. B. D. U. L., Hore, M. A.Y. K. H., Chakraborty, K .U. U. S. H. I. K, & Roy, S. U. B. H. R. A. J. Y. O. T. I. Phytochemical analysis and antioxidant activity of methanolic extract of leaves of *Corchorusolitorius. International of Current Pharmaceutical Research,*(2017);59-63.
25. Subramanya, M. D., Pai, S. R., Upadhya, V., Ankad, G. M., Bhagwat, S. S., & Hegde, H. V. Total polyphenolic contents and in vitro antioxidant properties of eight Sida species from Western Ghats, India. *Journal of Ayurveda and integrative medicine,*(2015); *6*(1), 24.
26. Kumar, S. P., Singh, N. P., & Mayur, P. In-vitro antioxidant activity of *Gossypium herbaceum* Linn. *International Research Journal of Pharmacy,*(2011);2 (7), 166-170.
27. Gbadamosi, I. T., Abiade, A. A., & Agbatutu, A. An Assessment of the Nutritional, Phytochemical and Antioxidant Properties of. *African Journal of Biomedical Research,*(2018); *21*(3), 33

Table 1. Percentage of Yield of *Berrya cordifolia* Leaf Extracts.

Sample	Leaf
Methanol	11.94%
Di-ethyl ether	1.45%

Table 2.Nature of the Crude Extract of *Berrya cordifolia* Leaf

Sample	Odour	Colour	Consistency
Methanol	Pungent	Dark green	Sticky
Di-ethyl ether	Pungent	Dark green	Sticky

Table 3.Phytochemical Analysis of *Berrya cordifolia* Leaf

Sr. No.	Phytochemicals	Test	Results	
			Methanol	Di-ethyl ether
1	Alkaloids			
		Mayer's Test	+	-
		Wagner's Test	+	-
		Hager's Test	+	+
		Dragendroff's Test	+	+
2	Carbohydrates			
		Molisch's Test	-	-
		Fehling's Test	-	-
		Barford Test	-	-
		Benedict's Test	+	+

3	Glycosides			
		Borntrager's Test	+	+
		Keller-Killani Test	-	+
s4	Proteins			
		Millon's Test	+	-
		Biuret Test	-	-
5	Phenols			
		Ferric chloride Test	-	+
6	Tannins			
		Lead acetate Test	+	+
		Ferric chloride Test	-	+
		Folin-Ciocalteu	+	-
7	Flavonoids			
		Alkaline reagent Test	+	+
		Lead Test	+	+
8	Saponins			
		Forth Test	-	-
9	Terpenoids			
		Copper acetate	+	+
		Salkowski's Test	+	-
10	Cardiac glycosides			
		Legal Test	+	+
11	Steroids			
		Salkowski's Test	+	-
		Liebermann-Burchard's Test	-	+

+ = Presence; **- =** Absence

('+' sign indicates the presence of the phytochemicals while '-' sign indicates the absence of the phytochemicals in the plant extract)

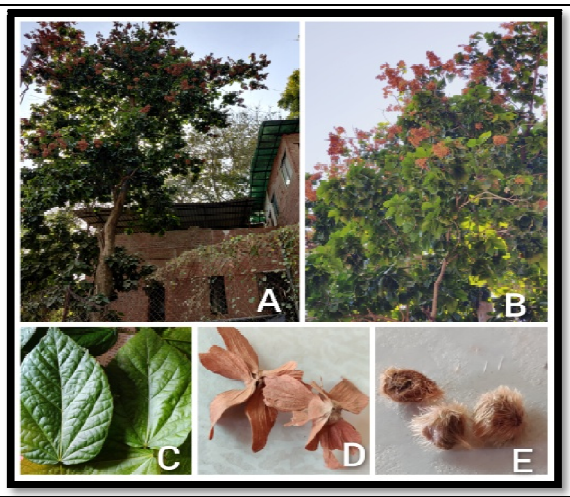

Figure 1: *Berrya cordifolia* (Willd.) Burret A Tree. B Flowers on tree. C leaves. D Flowers. E Hairy seeds.

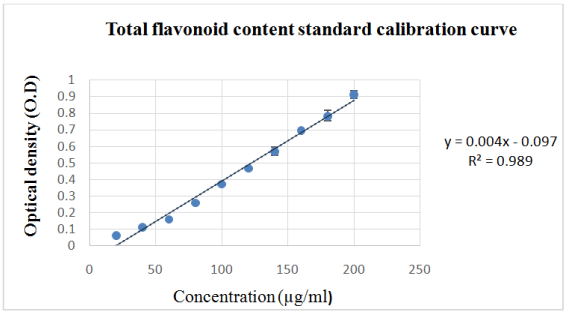

Figure 2: Standard curve of different concentration (µg/ml) of Gallic acid and their respective optical density.

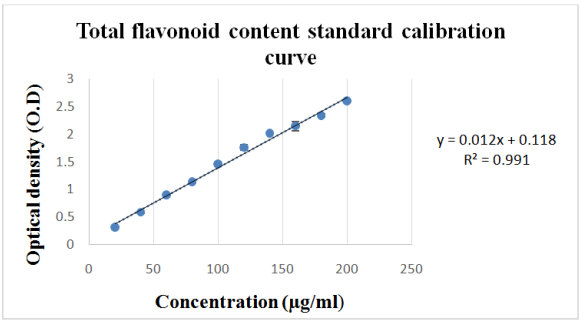

Figure 3: Standard curve of different concentration (µg/ml) of Quercetin and their respective optical density.

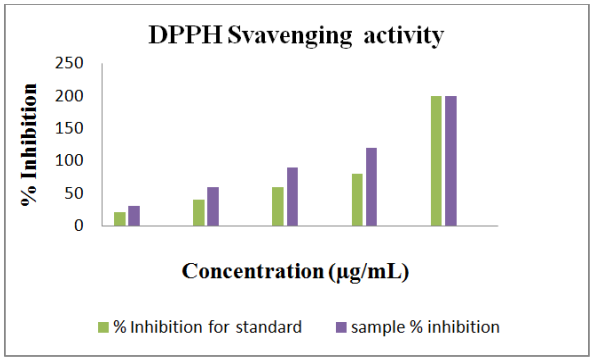

Figure 4: Inhibition activity of Ascorbic acid standard and methanolic leaf extract of *Berrya cordifolia*.

-Shows the percentage inhibition for the standard,

-Shows the percentage inhibition for the plant sample.

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RESEARCH ARTICLE

Impact of Social Media in Promotion of Organic Food Products

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ABSTRACT

In today's world, people are very sensitive to their health issues, and often take precautions to make sure they live a healthy lifestyle, which results in the increased demand of organic food products that are prepared and processed without using any chemicals. India is a democratic nation, where social media (Face Book, Whatsapp, Twitter, Instagram) is a vibrant tool to connect and share our thoughts. In this paper descriptive research design is used to collect 170 data. Also, for data analysis the descriptive statistics and Chi-Square tool is effectively used. Today, social media is the largest source of entertainment, this study analyse the role of various social media tools as a channel to develop a healthy nation. Therefore, this paper highlights the consumer's awareness towards organic foods products, and analyzes the role and impact of social media in promoting the organic products.

Keywords: Consumer awareness, Organic food products, Social media, E-commerce, Digital marketing

INTRODUCTION

In the yester years, lots of changes has been noticed in the preferences and consumption of people with the rising concern on the quality of food products as excessive use of chemicals were being used in the farms thereby deteriorating the air quality ,polluting the environment and resulting in land losing its fertility. The excess use of fertilizers and chemicals in growing food products has brought loss to human health and safety. In this study, the role of social media in promoting organic products, brings out the essence of healthy life by using the social media as an effective tool. Organic food products refer to healthy products made up of natural ingredients without using any kind of chemicals. Social media is the right tool for mass communication. In this digital world, everyone is active in social media as it is the fastest medium of disseminating information and attracts the attention of people.

LITERATURE REVIEW

Anwesha Chattopadhyay, Priyanka Khanzode (2019) undertook the study to understand the level of awareness about organic food products in Bengaluru and to understand the consumption pattern of individuals in Bengaluru. It was found that the consumption of organic food was raised in last five years of the study. People were aware of organic food and their benefits. People spent 27% of their budget on food on an average for organic food and rest on non-organic. Only 17% of all consumers were found to depend on organic food products totally. In fact, very few people are found to be dependent on such products for more than 10 years. The study suggests to advertise and undertake programs to create more awareness about the positive effects of using organic products.

D.T. Venkatakrishnan (2017) has made an attempt to find out the consumer's awareness towards organic food. It was found that there was a significant association between gender, age, educational qualification, monthly income and consumer awareness towards organic food products of the respondents and also felt the need for educating the consumers and creating awareness for the organic goods. The study reports that consumer awareness plays a vital role in determining the buying behavioural aspect for selecting organic food.

Md Tareq Bin Hossain and Pei Xian Lim (2016) evaluated the current status of consumer's buying behaviour towards organic foods in the emerging market. The study found that government support and policy, perceived beliefs and attitudes, knowledge and availability have a significant positive relationship with consumer behaviour towards organic foods. However, health consciousness, environmental concern and price do not have any significant relationship with consumer behaviour towards organic foods.

STATEMENT OF THE PROBLEM

People are very sensitive to their health issues nowadays and often take precautions to make sure they remain healthy. Organic food growers do not use antibiotics or chemical fertilizers, in their processes which might not affect the human body in negative ways. In this context it is very important to know the consumer awareness towards organic food products and role of social media in promotion of such products.

OBJECTIVES OF THE STUDY

1. To determine the consumer's awareness towards organic food products.
2. To analyse the role of social media in promotion of organic food products.

HYPOTHESIS

1.There is no significant difference in education and awareness of organic food products among consumers.
2.Social media play a vital role in promotion of organic food products.

DATA ANALYSIS

Table :1 Education and awareness of organic food products among respondents
Table-2 Role of Social Media in Promoting Organic Food Products

RESULT

The critical value $\chi 2$ at 0.05 level of significance from the table is 9.49 for degree of freedom 4. The calculated value of Chi -Square is 1.073 which is less than the table value, hence the hypothesis is accepted. It is concluded that there is no significant difference between education and awareness of organic food products amongst people. For 4 and 30 degree of freedom and 0.05 significant, the f table value is 2.689 .The calculated value is 0.1648 which is less than table value, hence hypothesis is accepted. It is concluded that social media plays a vital role in promotion of organic food products.

Findings of the Study

There is no significant difference between education and awareness of organic food products amongst people. Majority of the educated people fall in the age group 18-29 years are under/post graduates (46%) followed by professionals (16.2%) are well aware of organic food products available in the market. Majority say, 52% of respondents believes that social media plays a vital role in promotion of organic food products (facebook, Instagram, Twitter, LinkedIn, You tube) and has become an effective marketing tools for organic farmers in current scenario with the change in technology from traditional marketing tools to the creative digital tools whereas, 47% of them are aware of such products through their friends, relatives, T.V advertisements, family, books and grocery stores. Majority of the respondents (33.3%) buy organic food products from departmental stores , 25.3% of them buy directly from farmhouse, 24.2% of the consumers buy from retail stores followed by 17% of them prefers to buy such products online.

CONCLUSION

It is evident from the study that people are aware of organic food products and its consumption has raised in past few years as organic food is non-toxic and will enhance and nourish the health of people consuming it. Even in this covid -19 pandemic situation, our country able is to sustain only due to our traditional organic food products. It is clearly identified through this study that the awareness of organic food products is increasing day by day with the help of social media. Social media plays the vital role to develop a healthy nation. Organic farming practices are also one of our government initiatives on human health and should be promoted through advertisements to create more awareness about the positive effects of consuming such food items due to the fact that people do consume organic food ,but they also prefers to buy non- organic food. Therefore, people can be attracted and influenced to buy organic food products if the same is conveniently available at reasonable price.

REFERENCES

1. Anwesha Chattopadhyay ,Priyanka Khanzode -An Empirical Study On Awareness And Consumption Pattern Of Organic Food In Bengaluru City, The It Capital Of India: An Analysis With Respect To Different Demographic Factors And Availability Of Organic Food Products In Bengaluru, *International Journal of Research – GRANTHAALAYAH,* VOL 7 Jan 2019 ISSN- 2350-0530(O), ISSN- 2394-3629(P)
2. D. T. Venkatakrishnan- A Study on Consumers Awareness Towards Organic Food Products with Special Reference to Tirupur District , *International Journal of Applied and Advanced Scientific Research (IJAASR)* Impact Factor: 5.255, ISSN (Online): 2456 - 3080 (www.dvpublication.com) Volume 2, Issue 2, 2017 169
3. Md Tareq Bin Hossain and Pei Xian Lim -Consumers' Buying Behavior towards Organic Foods: Evidence from the Emerging Market, *Malaysian Management Review* July-December 2016 Vol. 51 No.
4. H.M. Chandrashekar -Consumers perception towards organic products-A study in Mysore city, *International Journal of Research in Business Studies and Management*,Vol-1, Issue 1 2014 pg-52-67

Table 1: Education and awareness of organic food products among respondents

Educational Qualification	Yes	No	Total (N=99)
Upto +2	16	1	17
Under Graduate/Diploma	33	3	36
Post Graduate	31	3	34
Ph.D.	5	1	6
Others	5	1	6
Total	90	9	99
Chi-square $\chi 2 = 1.073$ p-value is .898526		df =4	

Source- Primary data

Table 2: Role of Social Media in Promoting Organic Food Products

Social Media	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
FaceBook	3	2	4	4	4
Instagram	5	4	6	6	6
Twitter	1	0	1	1	1
LinkedIn	0	0	0	1	1
Youtube	0	0	0	1	1
Others	9	8	10	10	10
Total	18	14	21	23	23

Source: Primary Data

Table 3: Calculation of data using Analysis of Variance (ANOVA) test

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	7	36	5.142857	42.47619		
Column 2	7	28	4	28		
Column 3	7	42	6	57		
Column 4	7	46	6.571429	63.61905		
Column 5	7	46	6.571429	63.61905		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	33.6	4	8.4	0.164891	0.954523	2.689628
Within Groups	1528.286	30	50.94286			
Total	1561.886	34				

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RESEARCH ARTICLE

Antioxidant Activity of Some Fungi Collected from Marine Soil Sample of Thiruppullani Ramnad District,Tamil Nadu, India

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ABSTRACT

The intention of this work was to screen fungi isolated from marine soil in Thiruppullani, Ramanathapuram District, Tamil Nadu, India. The marine derived fungi as a model for bioactive exploration for new entities with antioxidant capacity. Four isolates of marine fungi were isolated. All fungal isolates showed antioxidant activities, isolate, indicating that marine fungal extracts can be considered promising tool in antioxidant drug industries. Marine derived fungi are considered a promising source of novel drugs due to their biodiversity and consequent chemo-diversity. Although marine microorganisms especially fungi are not well defined taxonomically, making this a promising frontier for the discovery of new medicines. Three in vitro assays were used to investigate the bioactive antioxidant potentiality of fungal extracts. The antioxidant activity four selected strains of *A.flavus, A.luchuensis, C.lunata, F.solani* showing quantitatively best antioxidant activity by Hydrogen peroxide scavenging assay, reducing power assay and TBA methods. The four selected strains of microorganisms show the best antioxidant activity by 500 µg/ml. Further work is needful to isolate the extract compound that is responsible for antioxidant activity and anticancer activity will be done in the future recorded the highest antioxidant activity (0.60±0.20) in reducing power assay, while *A.flavus* came first by recording. Hydrogen peroxide assay compared with ascorbic acid (0.13±0.04). In no inhibition assay, thiobarbutaric acid showed comparing with ascorbic acid. The preliminary result of our extracts, we can consider the marine derived fungus extracts as promising antioxidant drug candidates.

Keywords: antioxidant activity and marine fungi.

INTRODUCTION

The marine environment covers more than 70% of Earth's surface and represents the largest ecosystem on Earth, characterized by extremely variable and hostile physico-chemical parameters (low temperature, limited light access, high salinity, and high pressure). The world's oceans and seas account about 90% of the living biomass of our planet, dominated by unicellular microorganisms. In order to face the wide range of ecological niches that characterize the oceans, marine microorganisms have evolved across the evolutionary time scale, unique metabolic and genetic adaptation that led to extraordinary secondary metabolites diversity, with unmatched structures (Blunt *et al.,* 2018 and Bhatnagar and Kim, 2010). Recently, it was even understood that many bioactive molecules previously isolated from marine macroorganisms, are actually produced by associated microorganisms. Moreover, the fraction of Food and Drug Administration (FDA) approved marine natural products has also become enriched with microbial compounds, which represent a significant portion of approved antibiotics (Patridge *et al.,* 2016). Although microorganisms have an essential role in the ecosystem functioning, and global biogeochemical cycles of the major elements, we still know little about marine microbial diversity, the ecological role in the ecosystem and their metabolic capabilities.

The development of genomic approaches made possible to identify marine microorganisms, allowing scientists to understand the full extent of microbial biodiversity and functions in the world's oceans (Giordano *et al.,* 2018). Marine microbial eukaryotes, among which fungi, are abundant and ecologically important members of marine microbiota, and many studies, based on molecular and metagenomic approaches, have documented their once- unimaginable diversity, even in deep-sea hydrothermal ecosystems (Le Calvez *et al.,* 2009). In fact, it is noted that marine fungi are widely distributed in marine environments, from shallow water to deep sea, even down to the polar ice covers. They are found in sediments and in all kinds of living and dead organic matter. Cultivation- dependent studies demonstrated the huge abundance of fungi in marine macroorganisms, like sponges and algae. It has been observed that most marine sponges harbor often fungi with representatives of *Acremonium, Aspergillus, Fusarium, Penicillium, Phoma*, and *Trichoderma* (Wiese *et al.,* 2011). Moreover, thanks to the accumulation of fungi in sponges, these macroorganisms have been also used to find fungi belonging to the less common taxa, such as Beauveria, Botryosphaeria, Epicoccum, Tritirachium, Paraphaeosphaeria, Bartalinia and Volutella.

The marine fungi represent an important opportunity for bioprospecting, as the exploration of their potential in terms of the exploitation of molecules is still at an early stage. In order to evaluate the metabolic potential of marine fungi, several genomic sequences of fungi are currently under way. The already completed sequences showed a huge biosynthetic capacity of fungi, with generally approximatively 30 to 40 biosynthetic gene clusters coding for secondary metabolites in every genome. Therefore, marine fungal biotechnology represents an exciting and promising area of investigation (Luna, 2015), that is increasing. Recently, the number of new marine natural products is definitely high, e.g., in 2016 alone, 1277 new compounds with promising biomedical applications were identified, and 36% of these molecules are produced by marine fungi. The Free radicals and oxidants have a dual role in the body, as they can be both toxic and beneficial compounds. They are produced during the normal cell metabolisms, but also induced by external sources, e.g., pollution, cigarette smoke, radiation, medication. When these molecules are gradually accumulated, due to the imbalance between reactive oxygen species (ROS) generation and antioxidant defence systems, they generate oxidative stress. The ROS family includes numerous types of molecules that cause harmful effect towards cellular constituents. They react with most biological macromolecules (lipids, proteins, and nucleic acids) causing their degradation and destruction, with different huge consequences on cell biology. Several evidences in experimental and human trials indicate that oxidative stress play an important part in the pathogenesis of various chronic and degenerative processes, such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, inflammation, cardiovascular and neurodegenerative diseases (Ahmad, *et al.,* 2017 and Pham-Huy, 2008).

Moreover, lipid oxidation continues to represent a major problem in food industry. It may occur in foods during harvesting, processing and storage, destroying organoleptic properties (with the development of repugnant flavours and odours), or valuable nutrients (e.g., essential fatty acids, lipid-soluble vitamins, and proteins). producing toxic compounds, thus making the lipid or lipid-containing foods unsuitable for consumption (Madhavi *et al.,* 1995). In particular, lipid oxidation is also responsible for quality deterioration of muscle foods and dairy products, as well as fruits and vegetable crops (Kanner, *et al.,* 2007). The antioxidant effects is a promising strategy for prevention and treatment of these processes. For this reason, nowadays, there is increasing interestto study the properties of agents currently present, and to investigate the possibility of using novel antioxidant compounds. This antioxidants produced by marine fungi so far. We first deal with antioxidants deactivation mechanisms of reactive species and with the available assays to detect them and then we discuss the molecules with antioxidant activity, and the extraction methodologies, evaluating their potential applications in different application fields.

MATERIALS AND METHODS

Study Area

Marine soil samples were collected seasonally from Thiruppullani, Ramanathapuram (District), Tamil Nadu, India for a period of one year from May 2017 to April 2018.

Collection of soil sample

The sediment soil samples were collected from Thiruppullani, Ramanthapuram District, Tamil Nadu, India. The collected soil samples were brought to the laboratory in sterilized zip-lock polythene bags handpicked air dried and stored in containers for further analysis. Soil samples were collected from the study site at random during the four seasons in the year of May 2017 to April 2018.

Antioxidant activity

Hydrogen Peroxide Scavenging Capacity (Ruch *et al.*,1989)

The ability of the culture filtrate of *Aspergillus flavus, A.lucheunsis, Curvularia lunata* and *Fusarium solani* to scavenge hydrogen peroxide was determined according to the method of solution of hydrogen peroxide (40 mm) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both culture filtrate and standard (ascorbic acid) compounds were calculated: % of Scavenged [H2O2] = [(AC – AS)/AC] x 100

Reducing power assay (Oyaizu,1986)

The reducing power of the culture filtrate was determined according to this method. One ml of the culture different concentration (100, 200, 300, 400 and 500 µg/ml) containing in 1ml of the deionized water mixed with 2.5ml of phosphate buffer (pH 6.6) and 2.5ml potassium ferrocyanide (1%). The mixture was incubated at 50ºC for 20 minutes and 2.5ml of TCA (10%) and centrifuged at 3000 rpm. The upper layer of the solution was mixed with 2.5ml distilled water and $FeCl_3$ (0.5ml). The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated the higher reducing power. The absorbance compared with the standard ascorbic acid. The percent increase in reducing power was calculated using the following equation.

$$\text{Increase in reducing power (\%)} = \frac{\text{A test - A std}}{\text{A}} \times 100$$

Thiobarbitaric Acid (TBA) Method (Sawarka et al., 2009)

TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100ºC), melonaldehyde binds with TBA to form a red complex that can measured at 532 nm and 2 ml of 20% Trichloroacetic acid and 2 ml of 0.67%TBA solutions were added to 2 ml of the mixtures containing the sample (0.2 – 0.8 microgram) prepared in the FTC (Ferric thiocyanate) method. This mixture was kept in water bath (100ºC) for 10minutes and after cooling to room temperature. Then it was centrifuged at 3000 rpm for 20 minutes. Antioxidant activity was based on the absorbance of the supernatant at 532 nm on the final day of the assay. Malondialdehyde used as a standard (concentration of 20µg).The percentage of antioxidant activity was calculated by following formula for TBA.

RESULTS AND DISCUSSION

The results that the fungi might produce more bioactive compounds apart from antibiotics and these findings will facilitate further studies to gain better understanding of bioactive metabolites production in fungi. A number of fungi, in particular mushrooms, have been known to possess good antioxidant activity. However, much work still needs to be done to explore filamentous fungi for antioxidant activity and the results shown by *Aspergillus fumigatus* support this contention (Yen and Lee 1996). Antioxidant activity, as assayed by different methods, demonstrated static culture of *Aspergillus fumigatus* to be more suitable as compared to shake flask culture. This supports the earlier observation of various researchers who have used static conditions (Harper *et al.,* 2003) or low RPM (between 100 to 150 RPM) (Huang *et al.,* 2007). It might be attributed to a low amount of phenolic compounds produced under shaking conditions, which have been held responsible for antioxidant activity of fungi. Ten days of incubation period was optimum for antioxidant activity, and the subsequent decline in bioactivity could be due to the exhaustion of nutrients available for the fungi. This decline may also be attributed to the degradation of secondary metabolites (phenolic compounds) already produced by fungi as supported by decline in the phenolic content. The comparison of antioxidant activity of the cell-free culture broth obtained from the fungus grown at different temperatures revealed 25°C to be the optimum temperature, which correlate positively with its phenolic content, will be helpful in biotechnological more production of bioactive metabolites in near future.

The Present study revealed that the invitro experiments including free radical scavenging activity reducing power assay demonstrate that different soil fungi may have significant antioxidant activities and phenolic compounds. This indicate the scavenging activity of *Aspergillus flavus* (31%), *A. luchuensis* (12.5%), *Curvularia lunata* (22.8%) and *Fusarium solani* (32.2%). This indicate hydrogen peroxide scavenging assay of *Fusarium solani* was the potent (32.2%) antioxidant producer having broad spectrum against various free radicals. Previous studies have shown the linear correlation between total phenolic content and antioxidant activity; total phenolic content of *Aspergillus fumigatus* correlated well with the antioxidant activity which is in consonance with earlier studies (Sultana, *et al.,* 2007). The extract obtained from *Aspergillus fumigatus* showed good activity against DPPH radical by neutralizing the free radical character of purple color DPPH, either by transfer of electron or hydrogen atom to yellow-colored diamagnetic molecule revealing hydrogen donating property of phenolic compounds present in the extract which can be supported by the positive correlation (r = 0.817) between the results of DPPH assay and TPC (Bounatirou *et al.,* 2007). The present investigation revealed the reducing power assay of *Curvularia lunata* showed best activity (49.82%). This reducing potential activity was C_{50} with half maximal inhibitory. Reducing power assay proves the potential of the phenolic compounds in the extracts to act as reductones that inhibit lipid peroxidation by donating a hydrogen atom thereby terminating the free radical chain reaction. Moreover, this reducing potential may be due to the di- or monohydroxy substitution in the aromatic rings that possess potent hydrogen donating ability (Zhao *et al.,* 2006).

The extracts also showed appreciable chelating activity of metals, as the transition metals such as ferrous ion can stimulate lipid peroxidation by generating hydroxyl radicals through Fenton reaction. The chelating activity for ferrous ion was assayed by the inhibition of formation of red-colored ferrozine and ferrous complex. There was positive-correlation (r = 0.819) between chelating activity and TPC. As evident from studies, the cell-free extracts are able to scavenge nitric oxide ion, and correlation with TPC was found to be positive (r = 0.813). Mostof the literature is available on antioxidant activity of plants and mushrooms, though some of the fungi are known to produce antioxidant activity. To the best of our knowledge, apparently this is the first systematic report on antioxidant activity of *Aspergillus fumigatus* demonstrated by different assay procedures and its optimization by

statistical methods. Under optimal condition, *Aspergillus fumigatus* showed 89.8%, 70.1%, and 74.2% scavenging effect for DPPH radical, ferrous ion and nitric oxide ion, respectively. The yield for TPC was 12.3 mg/mL and reducing power showed absorbance of 1.0 and 70.5% activity for FRAP assay. In the present investigation, the effect of antioxidant activity of thiobarbutaric acid method was carried out for *Fusarium solani* (45.3%) C_{50} with half maximal inhibitory activity. To best of our knowledge apparently this is the first systemic report on antioxidant activity of *Fusarium solani* demonstrated by different assay procedures and its optimization method by statistical method. Further investigation are needed to discover bioactive consitituents in the fungal isolates and different antioxidant acitivity of fungi and component analysis should be studied in the near future. According to (Rathis *et al.,* 2017) Minimum number of colonies was Kollenkode area represented due to nutrient contents of study site maximum *Aspergillus* genera were presented. So, the fungal diversity was more useful for the content of environmental conservation the ocean of the world is varying greatly in intertidal amplitude and salinity of the water, all features that was dramatically affect fungal diversity.

REFERENCES

1. Ahmad, K.A., Yuan Yuan, D., Nawaz, W., Ze, H., Zhuo, C.X., Talal, B., Taleb, A., Mais, E. and Qilong, D. (2017). Antioxidant therapy for management of oxidative stress induced hypertension. *Free Radic. Res.* 51: 428–438.
2. Bhatnagar, I. and Kim, S.K. (2010). Immense essence of excellence: Marine microbial bioactive compounds. *Mar.Drugs*8, 2673–2701.
3. Blunt, J.W., Carroll, A.R., Copp, B.R., Davis, R.A., Keyzers, R.A. and Prinsep, M.R. (2018). Marine natural products. *Nat. Prod. Rep*.35, 8–53.
4. Bounatirou,S., Smiti, S. and Miguel,M.G. (2007). "Chemical composition, antioxidant and antibacterial activitiesof the essential oils isolated from Tunisian Thymus capitatus Ho ff. et link," *Food Chemistry*, 105: 146–155.peroxidation," *Food Chemistry*, 89 (3): 403–409.
5. Giordano, D., Costantini, M., Coppola, D., Lauritano, C., Pons, L.N., Ruocco, N., di Prisco, G., Ianora, A. and Verde, C. (2018). Biotechnological applications of bioactive peptides from marine sources. *In Advances in Microbial Physiology; Elsevier: Amsterdam, the Netherlands*, 73: 171–220.
6. microspora possessing antioxidant and antimycotic activities," *Tetrahedron*, 59 (14) :2471–2476.
7. Huang,W.Y., Cai,Y.Z., Hyde, K.D.,Corke, H.and Sun,M. (2007)."Endophytic fungi from Nerium oleander L (Apocynaceae): main constituents and antioxidant activity," *World Journal of Microbiology and Biotechnology*,23 (9):1253–1263.
8. Kanner, J. (2007). Dietary advanced lipid oxidation end products are risk factors to human health. *Mol. Nutr. FoodRes. Int. Food Res. Int.* 51: 1094–1101.
9. Le Calvez, T., Burgaud, G., Mahé, S., Barbier, G. and Vandenkoornhuyse, P. (2009). Fungal diversity in deep- seahydrothermal ecosystems. *Appl. Environ. Microbiol.,*75, 6415–6421.
10. Luna, G.M. (2015). Biotechnological potential of marine microbes. In Springer Handbook of Marine Biotechnology; Springer: Berlin/Heidelberg, Germany. 651–661.

11. Madhavi, D.L. Deshpande, S. and Salunkhe, D.K. (1995). Food Antioxidants: Technological: Toxicological andHealth Perspectives; CRC Press: Boca Raton, FL, USA, 1995.
12. Oyaizu, M. (1986) Studies on Products of Browning Reactions: Antioxidative Activities of Product of Browning Reaction Prepared from Glucosamine. Japan Journal of Nutrition, 44, 307-315.
13. Patridge, E., Gareiss, P., Kinch, M.S. and Hoyer, D. (2016). An analysis of FDA-approved drugs: Natural productsand their derivatives. *Drug Discov*. 21, 204–207.
14. Rathis. R, Madhanraj. P and Senthiskumar. G. (2017). Determination of Physicochemical parameters and Diversity pattern of three different places of Kanyakumari district. *Int. J. Adv. Res.* 5(1). 1036-1039.
15. Pham-Huy, L.A.; He, H. and Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci.* 4: 89–96.
16. R.J. Ruch, S.J. Cheng, J.E. Klaunig Prevention of cytotoxicity and inhibition of intracellular communication antioxidant catechins isolated from Chinese green tea Carcinogenesis, 10 (1989), p. 1003.
17. Sawarkar, H.A, Khadabadi S.S, Wandhare M.D, Farooqui I.A, and Deokate U.A. ''the antioxidant activity of the leaves of Barleria Grandiflora Daiz (Acanthaceae)'' Journal of Herbal Medicine and Toxicology., 2009; 3(2): 63-66.
18. Sultana,B., Anwar, F. and Przybylski,R., (2007)."Antioxidant activity of phenolic components present in barks of*Azadirachta indica, Terminalia arjuna, Acacia nilotica,* and *Eugenia jambolana Lam*. trees," *Food Chemistry,* 104 (3): 1106– 1114.
19. Wiese, J. Ohlendorf, B. Blümel, M. Schmaljohann, R. and Imhoff, J.F. (2011). Phylogenetic identification of fungi isolated from the marine sponge *Tethya aurantium* and identification of their secondary metabolites. *Mar. Drugs*, 9: 561–585.
20. Yen G.C. and LeeC. A.(1996). "Antioxidant activity of extracts from molds," *Journal of Food Protection,* 59 (1) 2:1327– 1330.
21. Zhao,G.R.,Xiang,Z.J, Ye, T.X. YuanY.J. and Guo, Z. X. (2006). "Antioxidant activities of Salvia miltiorrhiza and panax notoginseng". *Food Chemistry*, 99(4): 767-774

Table 1: Effect of antioxidant activity of Hydrogen peroxide scavenging assay by potential fungi

Different Concentration (µg/ml)	Activity (%)				
	Standard (ascorbic acid)	*A.flavus*	*A.luchuensis*	*Curvularia lunata*	*Fusarium solani*
100	0.08±0.12	0.09±0.03	0.11±0.03	0.22±0.07	0.05±0.01
200	0.09±0.07	0.13±0.04	0.12±0.04	0.04±0.01	0.04±0.01
300	0.11±0.09	0.13±0.04	0.12±0.04	0.08±0.02	0.05±0.01
400	0.12±0.06	0.14±0.04	0.14±0.04	0.12±0.04	0.08±0.02
500	0.13±0.04	0.15±0.05	0.17±0.05	0.12±0.04	0.08±0.02
IC_{50}	8.32	8.31	12.5	22.8	32.2

IC_{50} - Half maximal inhibitory, standard mean ± error

Table 2: Effect of antioxidant activity of reducing power assay by potential fungi

Different Concentration (µg/ml)	Activity (%)				
	Standard (ascorbic acid)	*A.flavus*	*A.luchuensis*	*Curvularia lunata*	*Fusarium solani*
100	0.12±0.10	0.27±0.09	0.09±0.03	0.26±0.08	0.06±0.02
200	0.15±0.11	0.34±0.11	0.12±0.04	0.06±0.02	0.10±0.03
300	0.21±0.12	0.38±0.12	0.14±0.04	0.07±0.02	0.12±0.04
400	0.24±0.14	0.46±0.15	0.13±0.04	0.32±0.10	0.13±0.04
500	0.26±0.17	0.60±0.20	0.30±0.10	0.12±0.04	0.16±0.05
IC_{50}	27.72	12.27	23.78	49.82	32.04

IC_{50} - Half maximal inhibitory, standard mean ± error

Table 3: Effect of antioxidant activity of Thiobarbutaric acid by potential fungi

Different concentration (µg/ml)	Activity (%)				
	Standard (ferric thiocyanate)	*A.flavus*	*A.luchuensis*	*Curvularia lunata*	*Fusarium solani*
100	0.19±0.09	0.40±0.13	0.10±0.03	0.11±0.03	0.08±0.02
200	0.10±0.12	0.37±12.3	0.12±0.04	0.13±0.04	0.17±0.05
300	0.13±0.11	0.08±0.02	0.13±0.04	0.15±0.04	0.15±0.05
400	0.14±0.07	0.11±0.03	0.15±0.08	0.17±0.04	0.24±0.08
500	0.16±0.09	0.13±0.03	0.18±0.10	0.19±0.05	0.25±0.08
IC_{50}	12.09	32.6	43.5	34.6	45.3

IC_{50} - Half maximal inhibitory, standard mean ± error

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RESEARCH ARTICLE

Assessment of Landfill Leachate and its Potential to Contaminate the Ground Water Quality in Near the MSW Unlined Dump Site

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ABSTRACT

The existing study was centered on an assessment of the landfill leachate and its potential to contaminate the ground water quality in near the MSW unlined dump site Dubagga, Lucknow city, U.P. India. As universal fact that urban residents have need of ample variety of urban services together with water supply, sewage disposal and solid waste management etc. Most of cities the solid waste is stacked in an open dumps lacking of providing appropriate lining, resulting degradation in Ground Water (GW) stratum. Physico-chemical parameters along with heavy metals like Fe, Cu, Ni, Zn, and Mn were analyzed in GW samples and are tabulated. The concentrations of Ca, Mg, TA and TH were found to be in higher than their acceptable limits in the GW samples mostly near the landfill, likely representing that groundwater quality is being noticeably affected by leachate seepage. A contrast of physico-chemical parameters with World Health Organization (WHO) and Bureau of Indian Standards (BIS) signify that the occurrence of contaminants in GW predominantly close to the landfill area warns its quality and hence renders the linked aquifer are seems to be unreliable for human consumption for further uses. The current study demands for the appropriate execution of waste dumps in the particular zone of the city.

Keywords: Leachate contamination, physico-chemical parameters, heavy metal analysis, Dubagga Landfill.

INTRODUCTION

Due to prompt expansion of urban county, business and trade behavior of urban trend prompting by brisk population escalation and the change in end user lifestyle has produced heavy amounts of solid waste. Open dumps are the accepted and the most frequent way of disposing of municipal solid waste. Generally they are located wherever land is available, without respect to safety, health hazard and esthetic degradation. The waste is repeatedly stacked as high as equipment permits. In a few cases the waste is ignited and allowed to be on fire. In others, the waste is periodically leveled and compressed (Sabahi et al. 2009) The dumping of solid waste in wild way, landfills can create considerable threats on the flora and fauna (Dong et al. 2008). The most frequently reported threat to the human health from these landfills is from the use of GW that has been polluted by leachate (Jhamnani and Singh 2009). Leachate is formed when moisture enters into the garbage in a landfill, extracts contaminants into the liquid phase, and generate moisture content adequately high to start liquid flow. Leachate generation in a landfill as a outcome of the contact of water with solid waste (Lo 1996). Leachate from a solid waste dumping site is usually found to contain major elements like Ca, Mg, K, Nitrogen and Ammonia, trace metals like Fe, Cu, Mn, Cr, Ni, Pb and organic compounds like phenols, polyaromatic hydrocarbons, acetone, benzene, toluene, chloroform, etc. (Freeze and Cherry 1979). Approximately above 90% of the MSW generated in India is directly dumped on land in an inadequate method (Chatterjee). Landfill surrounding area has a greater chance of groundwater contamination because of the possible pollution source of leachate producing from the nearby landfill site. Such type of contamination of GW results in a significant risk to local groundwater stratum, user and to the natural environment. The impact of landfill leachate on the groundwater has given augment to a number of researches in recent years and got major importance due to radical growth in population (Saarela). A lot of approaches that can be used to examine the groundwater and contamination. It can be judged by either the experimental determination of the impurities or their estimation through mathematical modeling (Moo-Young et al.,) Quality of the municipal solid waste (MSW) depends on a variety of factors such as change in the life style, food habits, standard of living and cultural custom of population and ambiance. The quantities of MSW generation rate both in terms of per the day and per capita basis for seven most important metros cities as shown in Fig.1 accordance with SPCB Mumbai report.

MATERIAL AND METHODS

Site Specification

There are just about 23 new and old MSW dumping sites in Lucknow city among which Dubagga site has been preferred for study. Dubagga landfill lies at 26.47°North and 80.55° East, is situated at 180-meter distance in north direction of the Chandoia village near Musabag and western direction of Lucknow city is low lying area and close to the fish market and Kadimi Kabristan. It receives about 1000 Metric tons municipal solid waste per day. Fourteen years (2007) have been passed since dumping started at this site. Large areas near landfill are open so having chances of contaminant rapidly reach into the groundwater by mixing with rain water in the wet season (Fig 2).

Composition and Quantification of MSW

To access the composition and quantification of fresh MSW from the study area the four pits were excavated within landfill site of size 3'x3'x3' around and10kgs. sample size of waste was taken from each pit and total waste samples of 40 kg. were segregated and weighted just immediate taking from the pits at site by segregating and weighing them into seven different categories viz. PVC, glass, wood, iron, thermocol, cloth and leather and Inert etc. As per Table 1 and Fig 3

Samples Collection

A total of 42 samples of GW were collected from 07 locations within the study area to examine the severity of leachate in the GW. The physico-chemical, heavy metal and biological analysis was carried out according to the international standard methods (APHA 1998). A arbitrary sampling technique was adopted to collect the GW

samples with due concern to distinguish land-use patterns, topography, and areas close to dump site (Kale et al. 2010). GW samples of 42 numbers (24 hand pumps, 18 bore wells) were collected within 3.0 km from the landfill site. The essentials of groundwater sampling locations are given in Table 2. The geo-coordinates were obtained from the GPS camera. The groundwater sampling locations with the study area, approx. depth of sampling stratum along with distance from the landfill site is also marked in the table 2. Samples were collected from hand pumps and bore wells/ tube wells which were pumped for 15–30 min before procuring sampling (Andrew et al. 2011). These samples were collected in well cleaned P.V.C. Tarson bottles of 1L capacity after rinsing with the sample and sealed airtight to keep away from evaporation, stored at 4^0C and analyzed within 24 hours.

Analytical Methods

The analysis parameters were selected based on their comparative significance in MSW landfill leachates composition, and their pollution prospective on GW in particular area (Bagchi 2004). The physico chemical parameters like as Total Dissolved Solids (TDS), Total Alkalinity (TA), Total Hardness (TH), major Cations such as Calcium (Ca^{++}) and Magnesium (Mg^{++}), major Anion such as Chlorides (Cl^-) and Sulfates (SO_4^{--}) of the leachate and GW samples were analyzed by titrimetric methods. Reasonably Chloride was included in the water quality assessment because of its compute of extent of dispersion of leachates in groundwater sources (Chapman 1992). Sulfates (SO_4^{--}) in the GW samples was also analyzed by Nephelometric turbidity technique (APHA 2017, 23rd edition). Nitrates (NO_3^-) Amount of Chemical Oxygen Demand (COD) was obtained by closed reflux titrimetric method, even as Biochemical Oxygen Demand (BOD) was find out by oxygen determination by Winkler titration for the conserved leachate sample. The investigations in this study were repetitive 02 or 03 times until concordant values were obtained, and all the tests were carried out according to the standard methods (APHA 2017, 23rd edition). The data was statistically analyzed by setting up and calculating a correlation template for the a variety of parameters using Statistical Package for Social Sciences (SPSS) apps (Norusis 1997)and also MS Excel 2000. An Atomic Absorption Spectrophotometer (AAS) make by Thermo Fisher Scientific, USA with D2 background correction lamp used to analyzed heavy metals such as Fe, Cu, Mn, Pb and Zn etc. The concentrations in the ground water samples were analyzed using Standard solutions of heavy metals viz. Cu, Cd, Mn, Pb and Zn were ready with distill water along with Copper Sulfate ($CuSO_4.5H_2O$), Cadmium Sulfate ($CdSO_4.8H_2O$), Manganese Sulfate ($MnSO_4.7H_2O$), Lead Nitrate [$Pb(NO_3)_2$], and Zinc Nitrate [$Zn(NO_3)_2.6H_2O$]

RESULT AND DISCUSSION

Physico-chemical characteristics of ground water

In view of the fact that the studied area is currently used for urban and some extent of agriculture purpose as per Graphical analysis, indicates the desirable and max. acceptable suggested limit by Bureau of Indian Standard (BIS, 1991) and World Health Organization (WHO, 1997) the pH of all the GW samples was likely close to neutral, the range between 6.28 to 7.78 (Fig.4). The Electrical Conductivity (EC) is an important indicator of the quantity of material suspended in water. EC in the landfill area range between 511.33 and 1515.67μS/cm and was found to be moderate in general. A little high conductivity values observed for the ground water close to the landfill (GW1) is a sign of its effect on the water quality. Total Dissolved Solids (TDS) signs the universal nature of water quality or salinity. The value of TDS at all samples were reported in between 347.7 and 1030.7 mg/L (Fig.5). Four samples were non-saline and three samples were slightly saline (Table 5). This elevated value of TDS may be due to the leaching of a variety of pollutants into the groundwater. The increase concentration of TDS reduces the sweetness and may cause gastro-intestinal nuisance in human and may also have laxative effect mostly upon transits (WHO, 1997). COD is a gauge of oxygen equivalent for the organic matter content of the water susceptible to oxidation by a strong chemical oxidant and thus is an index of organic contamination. The level of COD in the GW samples is found in zero amount, indicating the no occurrence of organic contaminants in the water. The concentration of Total Alkalinity (TA) as CaCO3 in GW samples exists from 170 to 483.33 mg/ L (Table 5) The increases amount of alkalinity (samples 1,2, 3, 4, and 6) promotes water with objectionable taste, and may be injurious to human health more than

desirable limits of pH, TDS and TH. Multivalent cations, predominantly Mg^{2+} and Ca^{2+} are frequently present at a considerable concentration in natural waters. These ions are easily precipitate and in specially react with soap to make it hard to get rid of scum. Total Hardness (TH) is usually expressed as the total concentration of Ca^{2+} and Mg^{2+} in mg/L, corresponding $CaCO_3$. TH ranged from 183.33 to 511.33 mg/L (Table 5). Ca^{2+} concentration in GW ranges from 44.8 to 100 mg/L (Table 5). Ca^{2+} frequently received from carbonate-based minerals, like as calcite and dolomite. Ca^{2+} and SiO_2 may also come from the suspension of concrete in roads and the footpaths. The concentration of Ca^{2+} got to be high in samples from GW1. The concentration of Mg^{2+} ions differ from 17.33 to 63.5mg/L (Table 5). Mg^{2+} salts are cathartic and diuretic and high concentration may produce laxative effect, on the other hand low amount may cause structural and functional irregularities. The concentration of F^- in the concerned site water samples ranged from 0.20 to 0.33 mg/L and obtained to be allowable limit. F^- at low concentration (~1mg/L) in potable water has been measured advantageous but high concentration may results in the form of dental fluorosis i.e., tooth mottling and more dangerously (Ravindra and Garg, 2006). The concentration of NO_3^- in water sample ranges from 5.8 to 14.07mg/ L (Table 5) and recorded within the allowable limit. Concentration of SO_4^{2-} in water sample exists from 16.37 to 31.45 mg/L and was recorded within the permissible limit. (Table 5).

Heavy Metal Analysis

Ground water samples were analyzed for heavy metal like as Fe, Cu, Ni Zn and Mn which were characterized as objectionable metals in potable water. WHO (1997) has projected below than their desirable value of 0.26, 0.00, 0.00, 0.11and 0.00 mg/L respectively in drinking water, All heavy metals were found within their acceptable limit (Table 5) except Fe in the sample GW1 and GW2 and responsible to impart reddish grown stains on laundry and unpleasant taste, odor in association with manganese. Heavy metals stay put in the waste or at the waste–rock boundary as a result of redox administrated precipitation reactions (Yanful *et al*., 1988). Additionally the metal mobilization is also restricted by physical sportive techniques and landfills have an inherent in situ capacity for reducing the mobility of poisonous heavy metals (Pohland *et al*., 1993). This stabilization of heavy metals minimize the risk of direct deadly effects due to absorption of leachate contaminated groundwater. On the other hand, once the leachate leaves the site the condition changes. In general leachate is a strong reducing liquid produced under methanogenic environment and on coming into contact with stratum materials has the ability to reduce soared heavy metals in the aquifer prevailing conditions. The most essential reactions are the reduction of Fe and Mn to more soluble variety. Therefore the concentration of these mechanism increases under encouraging conditions close to a landfill and may promote to a severe toxic threat.

Correlation Analysis

Correlation is a mathematical analysis tool used to assess the extent of interrelation and association between two variables (Nair et al. 2005). A correlation of 1 shows a perfect positive relationship between two variables. A correlation of -1 shows that one variable change inversely with relation to the other. A correlation of zero shows that there is no link between the two variables (Kapil et al. 2009). Table 6 indicates the correlation matrix amongst the twelve GW quality parameters of the designated area. TDS shows strong correlation with major water quality parameters viz. TA, TH, Ca, Mg and Cl. It indicates the fair enough positive correlation. A few of the other highly important and positive correlation was found which also shows linear correlation. This suggested that presence of bicarbonate; chloride and nitrate in the study area greatly influence the TDS. pH was negatively correlated with all the sample parameters. In the same way, few other parameters were also found to have negative correlation, like TDS and Fluoride (r = -0.05819),

CONCLUSION

Physico-chemical parameters as well as heavy metals viz., Fe, Cu, Ni, Zn, and Mn were analyzed in GW samples and are recorded. The concentrations of Ca, Mg, TA and TH were found to be in more than their acceptable limits in the GW samples mainly near by the landfill sites, likely indicating that groundwater quality is being considerably affected by leachate penetration into the sub surface water. A contrast was carried out for the physico-chemical

parameters with World Health Organization (WHO) and Bureau of Indian Standards (BIS) shows that the occurrence of contaminants in GW chiefly near the landfill sites indicates its quality and thus renders the related aquifers are not to be trusted for future domestic water supply and other agricultural and commercial uses.

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REFERENCES

1. Ajai Kumar Rai, Brajesh Mishra (2016) et. al.: Geotechnical properties based characterization of closed dumpsite: A case study. 2347-6710 IJIRSET, Vol. 5, Issue 3, March 2016
2. Archana, Daoud Ali (2014) et, al.: Assessment of the status of municipal solid waste management (MSWM) in Lucknow – capital city of Uttar Pradesh, India, Journal of Environmental Science, Toxicology and Food Technology, e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 5 Ver. II (May. 2014), PP 41-49 www.iosrjournals.org www.iosrjournals.org.
3. APHA 23rd Ed. 2017, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington DC USA, 15
4. Barjinder Bhalla1, M.S. Saini, (2014) et. al.: Assessment of municipal solid waste landfill leachate treatment efficiency by leachate pollution index, International Journal of innovative research in science, engineering and technology, vol. 3, issue 1, january 2014.
5. Bhide, A.D., Shekdar, A.V.(1998) et. al.: Solid waste management in Indian urban centers. International Solid Waste Association Times (ISWA) (1), 26–28.
6. BIS (Bureau of Indian Standards) 10500:1991 (Reaff : 2012) Drinking water standards.
7. Butow, E., Holzbecher, (1989) et. al.: Approach to Model the Transport of Leachates from aLandfill Site including Geochemical Processes, Contaminant Transport in Groundwater. Kobus and Kinzelbach, Balkema, Rotterdam, pp. 183–190
8. Carpenter, P. J., Ding, A. (2012) et. al.: Identifying Groundwater Contamination Using Resistivity Surveys at a Landfill near Maoming, China. Nature, Education Knowledge 3(7):20.
9. Chakrabarty, P., Srivastava (1995) et. al.: Solid waste disposal and the environment – a review. Indian Journal Of Environmental Protection 15 (1), 39–43.
10. Christensen, J. B., D. L. Jensen (1998) et. al.: Characterization of the dissolved organic carbon in landfill leachate-polluted groundwater, Water Res., 32, 125-135.
11. Central Ground Water Board, New Delhi, 2003: Ground Water in Delhi-Improving the Sustainability through Rain Water Harvesting
12. Chatterjee R (2010) et. al.: Municipal solid waste management in kohima city-india. Iran J Environ health sci and eng. 7 (2): 173-180.
13. Central Ground Water Board, New Delhi & Central Pollution Control Board-Status of Ground Water quality and Pollution Aspects in NCT-Delhi, 2000
14. Central pollution Control Board, Status of Solid Waste Generation, Collection, Treatment and Disposal in Class – II Towns, Control of Urban Pollution Series: CUPS/50/1999-2000.
15. Central Pollution Control Board (CPCB), (2004) Management of Municipal Solid Waste. Ministry of Environment and Forests, New Delhi, India.
16. CPHEEO Manual on Municipal Solid Waste Management-2000, Published by Central Public Health & Environmental Engineering Organization (CPHEEO), Government of India.

17. CPCB (2000), Status of Solid Waste Generation, Collection, Treatment and Disposal in Metrocities, Series: CUPS/46/1999–2000.
18. Dubey, M. Chakrabarti (2016) et. al.: Landfill mining as a remediation technique for open dumpsites in India, International Conference on Solid Waste Management, 5IconSWM 2015 Procedia Environmental Sciences 35 (2016) 319 – 327
19. Fatta D, Papadopoulos A, (1999) et. al.: A study on the landfill leachate and its impact on the groundwater quality of the greater area. Environ Geochem Health. 21 (2): 175-190. 10.1023/A:1006613530137.
20. Flyhammar, P (1995): Leachate quality and environmental effects at active Swedish municipal landfill, in: R. Cossu, H. T. Christensen and R. Stegmann (eds) Regulations, Environmental Impact and Aftercare. Proceedings Sardinia '95, Fifth International Landfill Symposium. Vol. III, Sardinia, Italy, pp. 549–557.
21. G. Siva Praveena, P.V.V. Prasada Rao (2016) et al.: Impact of leachate on soil properties in the dumpsite (A case study of greater visakhapatnam International Journal of Engineering Research and General Science Volume 4, Issue 1, January-February, 2016 ISSN 2091-
22. Indian Standard for Drinking Water as per BIS specifications (IS 10500-2012) (Second Revision)
23. Manual on Water Supply and Treatment, third edition — revised and updated May 1999, Ministry ofUrban Development, New Delhi.
24. Mohammed Saidu (2011): Effect of Refuse dumps on ground water quality, Pelagia Research Library, Advances in Applied Science Research, 2011, 2 (6):595-599
25. Mohan S, Gandhimathi R(2009) et. al.: Removal of heavy metal ions from municipal solid waste leachate using coal fly ash as an adsorbent. J Hazard Mater.169 (1–3): 351-359
26. Mor S, Vischher A, Ravindra K, Dahiya RP (2006) et. al.: Induction of enhanced methane oxidation in compost: Temperature and moisture response. Waste Manage. 26 (4): 381-388. 10.1016/ j.wasman.2005.11.005.
27. Moo-Young H, Johnson B, Johnson A (2004) et. al.: Characterization of infiltration rates from landfills: Supporting groundwater modeling efforts. Environ Monit Assess. 96: 283-311.
28. Nitin Kamboj,(2013) et. al.: Impact of solid waste disposal on ground water quality near Gazipur dumping site,Delhi, Inda. International Journal of Applied Natural Science.,5(2):306-312.
29. Pervez Alam & Kafeel Ahmad (2013 et. al.: Impact of Solid Waste on Health and The Environment, Special Issue of International Journal of Sustainable Development and Green Economics (Ijsdge),Issn No: 2315-4721,V-2, I-1,2, 2013
30. Saarela J (2003) et. al.: Pilot investigations of surface parts of three closed landfills and factors affecting them. Environ Monit Assess., 84: 183-192. 10.1023/A:1022859718865.
31. Shaoli Dea, Biswajit Debnathb (2016) et. al.: Prevalence of health hazards associated with solid waste disposal- A case study of Kolkata, India, International Conference on Solid Waste Management, 5IconSWM 2015, Procedia Environmental Sciences 35 (2016) 201 – 208.
32. SW Jackson, Suite 320, Topeka (2013) et. al.: Leachate Sampling for Reduction and/or Termination of Postclosure CareTechnical Guidance Document S-2013-G3
33. Tauqeer Alam1*, Ankit Kumar2et (2018) et. al.: Recent Progress In Waste Management System Of Lucknow City: A Minor Review, Www.Confrenceworld.In Isbn : 978-93-87793-15-6, April 2018
34. United States Environmental Protection Agency (US EPA): (1984). Office of Drinking Water, A Ground Water Protection Strategy for the Environmental Protection Agency. 11.
35. Uma Pd. Pandey, Rajiv Banerjee et. al. (2016): Evaluation of Solid Waste Collection and Segregation: A Case Study of Integral University Campus, Lucknow volume 4, Issue 5, May (2016) www.ijeter.everscience.org
36. U. P. Pandey, M. A. Khalid, et. al. (2019): Paradigm shift in MSW treatment and disposal Technology-A review, Volume 6, Issue 1 (E-ISSN 2348-1269, P- ISSN 2349-5138) IJRAR February (2019) www.ijrar.org
37. W.H.O. Guidelines for Drinking Water Quality. 3rd Edition Vol. 1 Recommendations, 2008.

Table 01

S. No.	Waste type	Weight (in kgs.)	Weight (in %)	Round off%
1.	Inert (C&D)	25.870	64.675%	65%
2.	P.V.C. (Polythene)	7.620	19.05%	19%
3.	Cloth and leather	4.700	11.75%	12%
4.	Glass	0.900	2.25%	2%
5.	Wood	0.480	1.20%	1%
6.	Iron	0.174	0.435%	0.5%
7.	Thermocol	0.256	0.64%	0.5%
	TOTAL	40.000 Kgs.	100.00%	100%

Table 02: Hydro-geological summery of GW sampling stratums along with geographical position

Sl. No.	Sample Location (≤ 200 m. radius)	Source	Code	Geo-coordinates		Distance from Landfill	Depth of stratum
				Latitude^0 (N)	Longitude^0(E)	In KM	In feet
1.	Dubagga Crossing, Mall Road	H.P.	GW1	26°53'50.69"N	80°52'13.56"E	0.32	60-100
2.	Ghaila Village West	H.P.	GW2	26°54'0.24"N	80°52'47.57"E	0.67	60-100
3.	Bari Kalan, Vasant Kunj	H.P.	GW3	26°53'19.02"N	80°52'32.03"E	1.5	60-100
4.	Hari Nagar	H.P.	GW4	26°52'56.59"N	80°53'1.22"E	2.15	60-100
5.	Sabji Mandi, Balaganj Crossing	B.W.	GW5	26°52'30.96"N	80°51'45.49"E	2.75	160-200
6.	Chandoiya Village	B.W.	GW6	26°53'36.21"N	80°51'49.67"E	1.12	160-200
7.	Allu Nagar Diguria	B.W.	GW7	26°54'53.41"N	80°53'50.24"E	3.0	160-200

Table 3: Testing parameters and standard protocol for GW analysis

S. N	Test Parameters	Standard Protocol (APHA)	Detention range	INDIAN STANDARDS as per IS 10500:1991(Reaff:2012)	
				Desirable	Acceptable
1.	pH	Ref. 23rd Ed. 2017, 4500H+ A+B	2 to 12	6.5-8.5	No. Relax
2.	EC	Ref. 23rd Ed. 2017, 2510 A+B	5-20000	-	-
3.	TSS (mg/l)	Ref. 23rd Ed. 2017, 2540-D	5-1000	-	-
4.	TDS (mg/l)	Ref. 23rd Ed. 2017, 2540-C	5-10000	500.00	2000.00
5.	Alkalinity (mg/l)	Ref. 23rd Ed. 2017, 2320 A+ B	5-1500	200.00	600.00
6.	Total Hardness (mg/l)	Ref. 23rd Ed. 2017, 2340 A+C	5-1500	200.00	600.00
7.	Ca (mg/l)	Ref. 23rd Ed. 2017, 3500 Ca A+B	5-1000	75.00	200.00
8.	Mg (mg/l)	Ref. 23rd Ed. 2017, 3500 Mg A+B	5-1000	30.00	100.00
9.	Chloride (mg/l)	Ref. 23rd Ed. 2017, 4500 Cl A+B	5-1000	250.00	1000.00
10	Sulphate (mg/l)	Ref. 23rd Ed. 2017, 4500-SO42- E	1-250	200.00	400.00
11	Nitrate (mg/l)	Ref. 23rd Ed. 2017, 4500-NO3- B	5-100	45.00	No. Relax
12	Fluoride (mg/l)	Ref. 23rd Ed. 2017, 4500-C	0.05-10	1.00	1.50
13	Fe (mg/l)	Ref. 23rd Ed. 2017, 3500 Fe B	0.02-10	0.30	No. Relax
14	Cu(mg/l)	Ref. 23rd Ed. 2017, 3111 A+B	0.05-50	5.000	15.00
15	Ni(mg/l)	Ref. 23rd Ed. 2017, 3111 A+B	0.02-2	0.100	0.30
16	Zn (mg/l)	Ref. 23rd Ed. 2017, 3111 A+B	0.02-50	5.000	15.00

17	Mn (mg/l)	Ref. 23rd Ed. 2017, 3111 A+B	0.05-5	0.100	0.30
18	Phenolic Compounds (mg/l)	Ref. 23rd Ed. 2017, 5530 A+C	0.001-5	0.001	0.00
19	T. Coli (MPN/100ml)	Ref. 23rd Ed. 2017, 9221B+C	1.8	0	0
20	E.coli (MPN/100ml)	Ref. 23rd Ed. 2017, 9221 B+E	1.8	0	0

Table 4: Analysis of GW for various parameters

S. No.	Parameters	Sample Code							Average of samples (Mean)
		GW-1	GW-2	GW-3	GW-4	GW-5	GW-6	GW-7	
1	pH	7.44	7.55	7.72	7.32	6.28	7.43	7.78	7.36
2	EC	1515.67	946.00	699.17	688.50	547.00	786.00	511.33	813.38
3	TSS (mg/l)	1.90	2.08	1.70	1.99	1.81	2.09	1.65	1.89
4	TDS (mg/l)	1030.70	643.30	475.40	468.20	372.00	534.50	347.70	553.11
5	Alkalinity (mg/l)	483.33	219.33	211.33	246.00	170.00	210.67	170.00	244.38
6	Total Hardness (mg/l)	511.33	238.67	233.33	256.00	235.33	214.67	183.33	267.52
7	Ca (mg/l)	100.00	59.73	57.33	62.93	50.93	49.93	44.80	60.81
8	Mg (mg/l)	63.50	21.70	19.44	23.98	26.24	23.65	17.33	27.98
9	Chloride (mg/l)	81.67	33.00	19.00	35.67	22.00	41.00	19.33	35.95
10	Sulphate (mg/l)	31.45	23.85	19.31	29.48	17.52	22.93	16.37	22.99
11	Nitrate (mg/l)	14.07	7.33	7.02	6.58	7.89	11.72	5.80	8.63
12	Fluoride (mg/l)	0.30	0.23	0.22	0.27	0.26	0.33	0.20	0.26
13	Fe (mg/l)	0.41	0.36	0.13	0.23	0.19	0.21	0.26	0.26
14	Cu mg/l)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	Ni(mg/l	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
16	Zn (mg/l)	0.16	0.11	0.10	0.19	0.00	0.13	0.11	0.11
17	Mn (mg/l)	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	Phenolic Compounds (mg/l)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	T. Coli (MPN/100ml)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	0.00
20	E.coli (MPN/100ml)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	0.00

Table 5: Comparison of ground water quality parameters with Indian (BIS) and International (WHO) standards

N.	Parameters	Mini.	Max.	Average	Standard Deviation	BIS Stand. (Max. allowable limit)	W.H.O. Stand. (Max. allowable limit)	Sample's Name Exceeding Max. allowable limit	Undesirable effect
1	pH	6.28	7.78	7.36	0.504	6.5-8.5	6.5-9.2	-	
2	EC	511.33	1515.67	813.38	342.145	-	-		
3	TSS (mg/l)	1.65	2.09	1.89	0.176	-	-		
4	TDS (mg/l)	347.7	1030.7	553.11	232.671	500	250	1,2,6	Gastro-intestinal irritation

5	Total Alkalinity (mg/l)	170	483.33	244.38	108.797	200	500	1,2,3,4,6	Nausea, muscle twitching
6	Total Hardness (mg/l)	183.33	511.33	267.52	109.920	200	300	1,2	Scale formation
7	Ca (mg/l)	44.8	100	60.81	18.375	75	150	1	
8	Mg (mg/l)	17.33	63.5	27.98	15.944	30	200	1	
9	Chloride (mg/l)	19	81.67	35.95	21.919	250	200		
10	Sulphate (mg/l)	16.37	31.45	22.99	5.798	200	50		
11	Nitrate (mg/l)	5.8	14.07	8.63	3.060	45	0.5		Blue baby
12	Fluoride (mg/l)	0.2	0.33	0.26	0.046	1.0	1.5		Fluorosis
13	Fe (mg/l)	0.13	0.41	0.26	0.098	0.3	0.0	1,2	Vomiting Diarrhea
14	Cu mg/l)	0	0	0.00	0.000	-	-	-	
15	Ni(mg/l	0	0.01	0.00	0.005				
16	Zn (mg/l)	0	0.19	0.11	0.060	5.0	5.0		Gastrointestinal disorders,
17	Mn (mg/l)	0	0.01	0.00	0.004	0.1	-		
18	Phenolic Compounds (mg/l)	0.00	0.00	0.00	0.00	0.001	0.0		
19	T. Coli (MPN/100 ml)	0.00	0.00	0.00	0.00	0	0	-	-
20	E.coli (MPN/100 ml)	0.00	0.00	0.00	0.00	0	0		

Table 6: Pearson's correlation analysis for different Ground water quality parameters

	pH	TDS	Alkalinity	Total Hardness	Ca	Mg	Chloride	Sulphate	Nitrate	Fluoride	Fe	Zn
pH	1											
TDS	0.197429	1										
Alkalinity	0.149447	0.945911	1									
Total Hardness	-0.00638	0.922645	0.982948	1								
Ca	0.086044	0.935197	0.983933	0.983710634	1							
Mg	-0.08642	0.897842	0.959671	0.985311314	0.94141	1						
Chloride	0.082225	0.939765	0.954816	0.92667653	0.911306	0.938439	1					
Sulphate	0.135787	0.766496	0.801417	0.738856021	0.810079	0.686599	0.827257	1				

Nitrate	-0.05219	0.804761	0.772811	0.763700169	0.696121	0.824609	0.878082	0.564745	1			
Fluoride	-0.27065	0.462355	0.458119	0.436007971	0.386497	0.510756	0.652067	0.573657	0.813289	1		
Fe	0.194287	0.781841	0.695576	0.671367083	0.684717	0.668937	0.737347	0.594073	0.474901	0.161228	1	
Zn	0.651073	0.449049	0.511182	0.358082956	0.460966	0.28835	0.518343	0.760763	0.243939	0.281975	0.377131	1

Except pH all parameters are measured in mg/l.

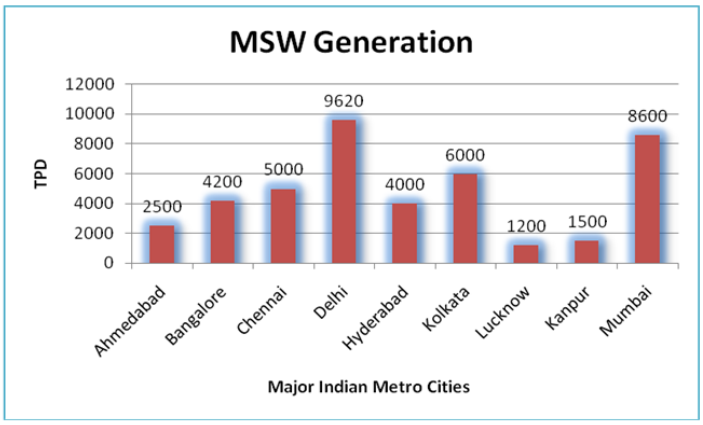

Fig 1: Source: SPCB, Mumbai Municipal Corporation-2016

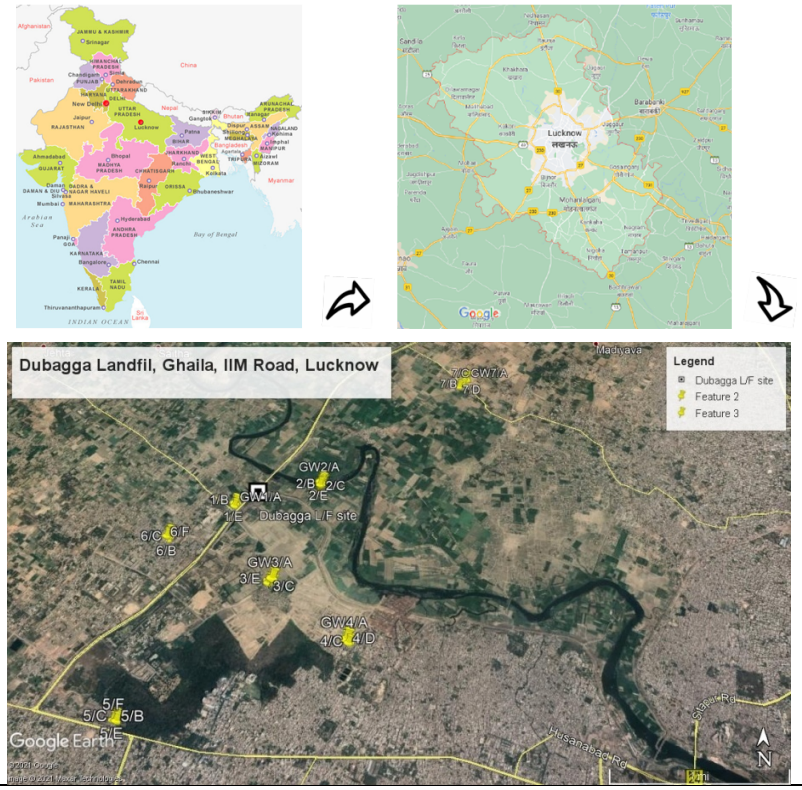

Fig 2: Source G. Earth: Satellite view of study area showing position of GW sample locations
Link- https://drive.google.com/file/d/16vbVST0AYV8y8uk-HkGev9X1i20hu_XG/view?usp=sharing

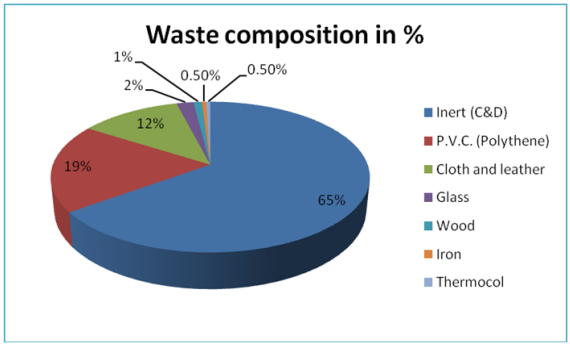

Fig 3: Composition of Landfill Municipal Solid Waste

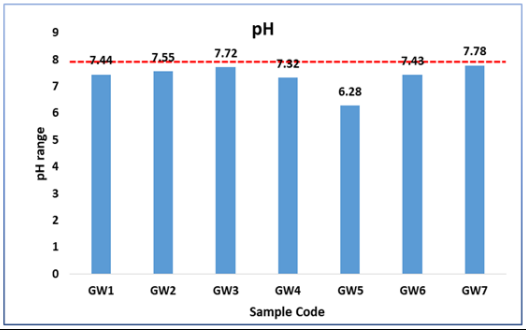

Fig 4

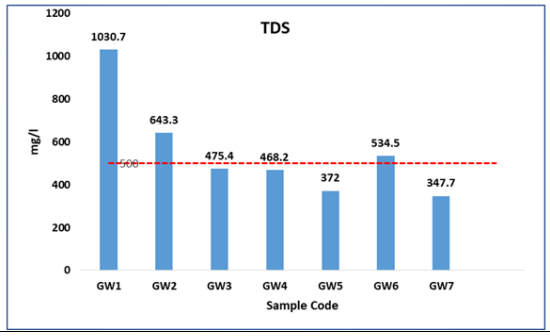

Fig 5

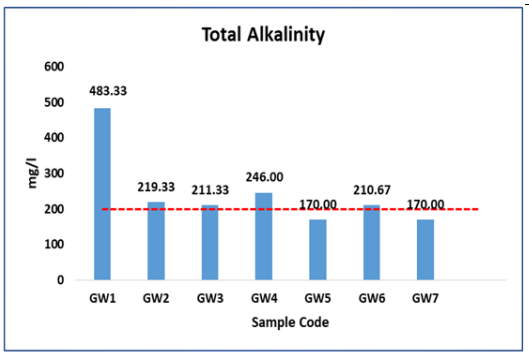

Fig 6

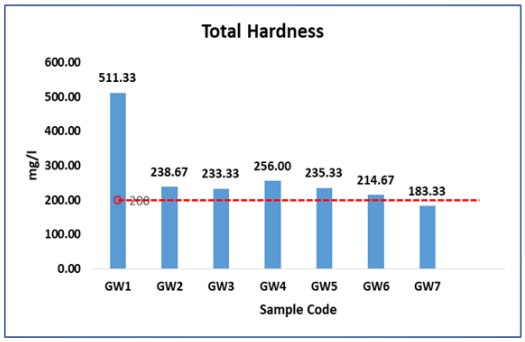

Fig 7

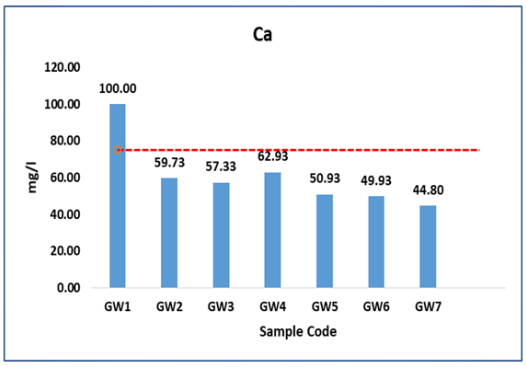

Fig 8

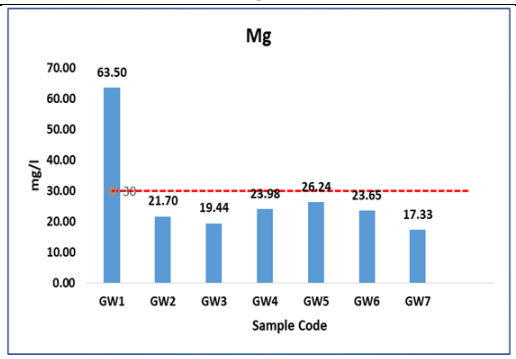

Fig 9

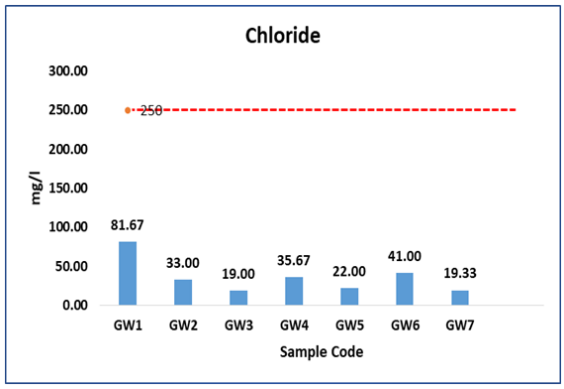

Fig 10

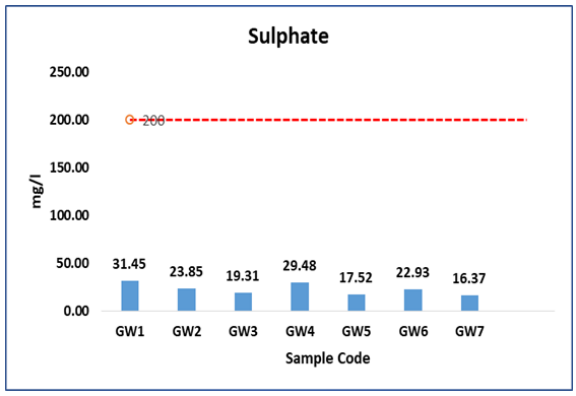

Fig 11

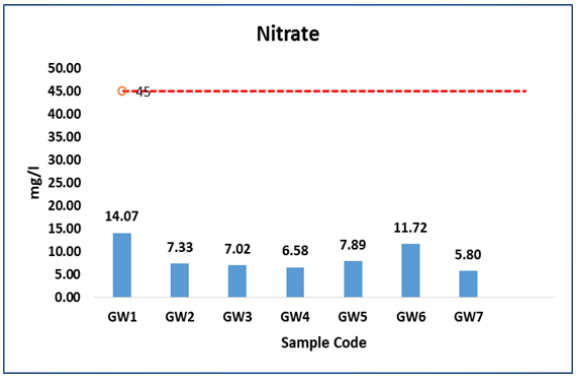

Fig 12

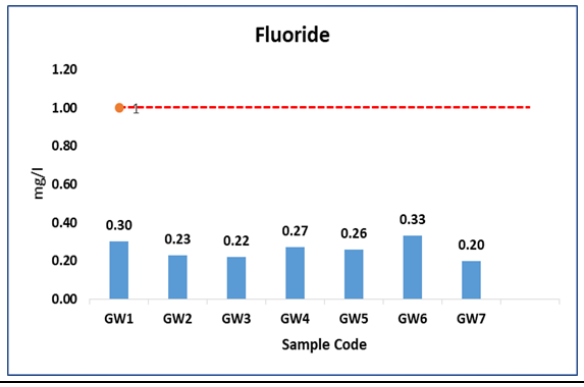

Fig 13

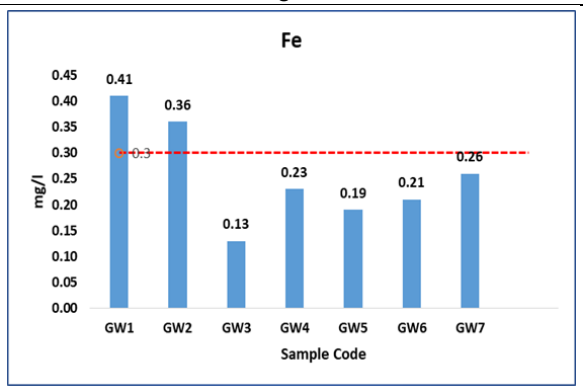

Fig 14

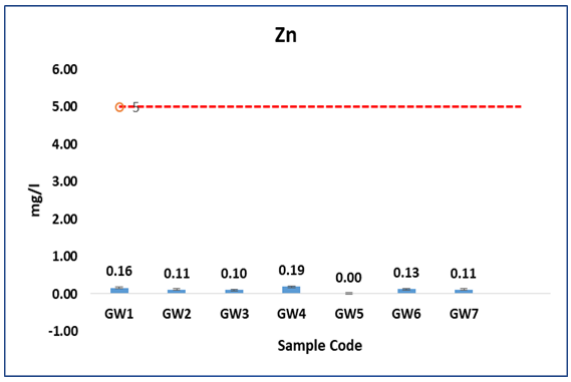

Fig 15

Graphical analysis of results showing permissible target line

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RESEARCH ARTICLE

E-Recruitment System

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ABSTRACT

E-recruitment system was designed to look into its efficiency, effectiveness, reliability, speed and its security. This study has been proven efficient, effectiveness, reliability, speed and its security. This study has been proven efficient as to tracking, filtering, facilitating selection process by providing an automated sorting based on the highest educational attainment. In addition, it provides effectiveness in reaching potential employees since this study was designed as an online system that is capable in reaching people around the globe. It also reliable in terms of managing records by the centralized storage that is capable in handling large amount of data. Moreover, it offered fast and less expensive process in distributing data by providing a notification mechanism. This study used constructive research design during the construction of the system. With this, the researcher enhanced the existing knowledge by filling in the gap that could satisfy system requirements. Likewise, the researcher used waterfall methodology in implementing SDLC process that served as a guide in building the system until the delivery period. In this process, the researcher followed the series of phases like planning, analysis, design and implementation. After thorough testing and evaluation made, this study is recommended to be implemented to every organization to improve its recruitment process. The researcher also recommended that additional enhancement must be considered like face-to-face interaction between applicant and HR personnel, and providing initial examination for every applicant. Before, implementing the system, the researcher also considered proper training for the administrator of the system to avoid misuse of it.

Keywords: E-recruitment system, selection process, online system, notification mechanism, constructive research design.

INTRODUCTION

Recruitment process is essential for every organization. It is when the establishment seeks and searches additional potential employees not just for the expansion of its population but also for its growth, development and productivity. Moreover, this process includes identifying applicant's skills, knowledge and expertise to be fitted for the vacant position. Through this, the applicants can be filtered and sorted based upon the level of competence. As Masters, et al (2010) stated that recruitment is defined as the process of creating a pool of potential candidates from which a selection process can refine the pool to the right talent to fill vacant positions. The process of assessing jobs in an organization includes determining the required skills and qualifications and the competency levels. HR Personnel make numbers of improvements in the area of recruitment and selection of candidates over the years. They applied different methods in advertising the vacancies, afterwards, they would find it time consuming and expensive. In addition, they find it more complicated especially in tracking the previous applicants, wasting substantial time in entertaining and interviewing unqualified candidates. Rahul (2012) emphasized that Labor intensive components of hiring like pencil and paper tests, job previews and interviews were used vastly in traditional recruiting. The traditional process has been fraught with delays of mis-communications and activities which resulted in high cost of hiring. With this, E-Recruitment System is being proposed in order to satisfy the needs of the organization, for it provides an easy way of finding, attracting, assessing new personnel via internet. This emphasizes on managing the entire processes such as providing initial filtering, enticing, searching an appropriate applicants. Online recruitment is a continuous process. In online recruitment, all process takes place concurrently. Online recruitment, when compared with traditional recruitment, saves time and cost for an organization, thereby improving its productivity, efficiency and effectiveness. It has a wider scope-locally, nationally and internationally-for enlarging the candidate pool and for improving the diversity of applicants. Consequently, employers have a better chance to find the right candidates for their vacancies (Barber, 2008).E-Recruitment System is designed to satisfy the needs of both HR personnel and applicant provided by the functionalities of the system. This online system provides wide range capacity of distributing data. Applicants have an opportunity to apply even without visiting the institution. Scrutinizing and retrieving applicant's papers would be easy and more convenient. Informing the applicant regarding vacancies, schedules for interviews and result of the interview is being done thru the use of the system via SMS.

Statement of Objectives

General Objective

To develop an E-Recruitment System that satisfies user requirements.

Specific Objectives

The following requirements are being laid down to determine the level of the system:

1. Effectiveness in reaching a large pool of potential employees.
2. Efficiency in tracking, filtering and facilitating selection process.
3. Reliability in managing applicant's and jobs records.
4. Speedy and less expensive data distribution process.
5. Security of the system.

Scope and Limitations of the Study

The scope and limitations of the study specifies the capacities and constraints of the system and specific consideration.

Scope of the Study

This project was conducted during the second semester, academic year 2013-2014 at Surigao State College of Technology. The system is capable in managing applicant's records. It can add, edit, and upload applicant's

information. It is also capable in managing job's records. It has adding, editing and deleting functionalities for job's information. Likewise, it can inform registered applicants for any vacancies based on their expertise, schedules for interview and receive the results of the application through SMS. Also, the system can sort applicants based on the highest educational attainment. In addition, the system is capable of uploading and downloading pertinent papers of the applicants.

Limitations of the Study

It cannot support monitoring of personnel's performance. Face-to-face interaction is not being catered as well. It doesn't also provide initial examination for the applicants.

Significance of the Study

This part of the study discusses the importance of developing a system to the following individuals and groups:

Applicant: The system may provide benefits for the applicant for it reduces time-consuming process of seeking and applying for a job.

Future Researcher: This system is subject for improvement in scope and functionalities, and somehow may be used for further research.

HR Personnel: The system is designed for HR Personnel for fast, effective, efficient and less expensive recruitment process.

Organization: The system is beneficial for the school for it reduces the cost and enhances the process of selecting the potential employees. It provides high-quality in managing applicant's records and distributing data.

Definition of Terms

For the clearer understanding of the terms used in this study, below are the meanings

Automated: It is the process of converting the traditional to computerized way.

E-Recruitment: It is the integration of the process of selecting the appropriate employee by utilizing the technology advancement.

HR: It stands for Human Resource. One of the offices of the institution that handles people who are currently working and accepting the applicants who want to be part of the institution.

SMS: It stands for Short Message Service. It is a system that is used to send message to mobile phones.

System: It is process of executing and achieving the goal through the proper organization of interconnected elements.

Traditional: It refers to the old way that includes paper-works and time-consuming process.

Conceptual Framework

A conceptual framework for a thesis acts as a visual outline to represent your concept or research idea. This framework expands upon the research problem as it relates to pertinent literature research (DGF, 2013). Figure 1 shows the flow of the study. This figure is composed of Input, Process, and Output. The first box shows the Input of the study that caters two interfaces. The first interface is for the administrator that includes job's information. The second interface is for the applicant that consists of the entry of Applicant's information, educational attainment as well as the contact number. Second box displays the Process of the study that includes validating data entry for both job's and applicant's information, identifying contact number of applicant and sorting process based upon the highest education attained by the applicant. Third box portrays the Output of the study which is the system that the researcher is expected to provide that is capable in keeping and managing records, filtering every application, and distributing information to every potential candidate through SMS.

METHODOLOGY

This chapter presents the project design used in this study. It shows how developmental research design and constructive design are being used effectively and in a useful way in constructing the system and helps the researcher to provide the user requirements. It also shows the Gantt chart schedule of activities.

Project Design

This system is a developmental research design wherein the study is designed, developed and evaluated according to the requirements of the user. It is being done incrementally. Based on the present situation, the development and enhancement of this study is iteratively developing. This system also is a constructive research where the researcher is constructing an E-Recruitment that aims to provide an enhancement based on the existing knowledge that would satisfy the system and user requirements. It applies System Development Life Cycle that guides the researcher during the construction of the system. In addition, the concept of waterfall development methodology is being used in order to properly apply and implement the theory of SDLC. It consists of four phases such as: planning, analysis, design and implementation. The four phases performs in sequence from one phase to the next.

E-Recruitment is an online system wherein it is designed to attract pool potential employees. The administrator is one of the HR Personnel who is assigned to add job vacancy as well as updating the database of the system; administrator also is responsible in monitoring the applicants as well as distributing information as to vacancies as well as interview schedules via SMS to every registered user. This system is an interactive system in the sense that it accepts inputs from the applicants such us personal information, they can also upload ready-made resume and application letter and can update their profile. Moreover, once the applicants are registered in the system, they can automatically receive message via SMS regarding job vacancies that are suited to their specification or the system gives result of their application. In addition, this system provides a well-organized and much secured process of keeping and retrieving applicant's information. It also capable in sorting applicants based upon their highest educational attainment.

Figure 3 shows the use case diagram of an E-Recruitment System. It shows that the administrator adds job description and its vacancies and monitor and manages the applicants. The applicant registers the system by inputting the personal information and updates the profile as well as searching vacant position. The system manages the inputted information such as applicant's information and job's information. It sends message via SMS to the registered applicants once there are vacant positions. This study has four modules such as: job's entry and applicant's entry, message module and applicant's records management module. Job's entry is where the administrator enters position title, salary grade, level, educational requirement, experience requirement, training requirement and the eligibility needed. Managing job's records also would be done in this module. Applicant's entry includes registration of the applicant's as well as managing their personal profile. This module also is where the applicant's resume and application letter is uploaded. Sorting of applicants information based upon their highest educational attainment is catered. Message module is where the administrator sends messages for any applicants for data distribution. Applicant's records management module is where the administrator manages the numbers of applicants. The administrator can delete or send message for that particular applicant. Table shows dynamic schedules of the researcher that lists all the tasks to be finished in an appropriate time.

RESULTS AND DISCUSSIONS

This chapter shows the description of the system and its corresponding requirements, software design, the development and testing and the implementation plan of the system. The interfaces of the system and its respective functions are also discussed in this chapter.

Description of the Project

The researcher chose to design E-Recruitment to reach more potential applicants around the globe. The system is being maintained by the HR Personnel who are also assigned to enter job's vacancy information such as position title, salary grade, level, educational requirement, experience requirement, training requirement and the eligibility needed. For the people who seek and search for a job, they should visit the site and view job vacancies. The applicant must register first before applying. They should provide their basic information including their highest educational attainment and upload their resume and application letter for later purposes. Once the registration is done, the registered applicants can apply for the posted vacant job. Next, is when the administrator views applicants for the specific job, the administrator notices that the sequence of the applicants is based upon the highest educational attained by them. Subsequently, the administrator can view and scan the resume and application letter of the applicant by downloading the files from applicant's profile. Once the administrator is interested for a particular applicant who seems to be qualified for the job, the system provides a notification mechanism through SMS that is capable in sending text message to the recipient. Moreover, the system also is capable in informing every registered applicant for any newly posted available job of the institution through SMS. Retrieving and managing records is more convenient and effective. This study produced output which can be considered as an E-Recruitment System, reaching more pool of potential and skillful people as well as a fast hassle-free data distributing process.

Requirement of the Project

Minimum hardware requirement

Processor: Intel Pentium Core to Dou 1.9 Ghz
Memory: 1 Gb DDR
Monitor: Cathode Ray Tube
Mouse: PS/2
Keyboard: PS/2 102 keys
AVR: 3 Gang for 220V

Software requirement

Operating System: Windows 7 Ultimate 32 bit
XAMPP
Language: PHP
Database: MySQL
Server: Apache

Design of Software

There are different considerations when it comes to design of software. Some of these aspects are:
Maintainability: Modifications and updating can be done to accomplish the user requirements.
Security: Proper security from any hostile practices can be accomplished.
Reliability: The software is capable in providing essential functions based upon the requirements.
Reusability: The software is able to cater additional features for enhancement and modification.
Usability: User-friendly interface is attainable for the user's benefits.

Screenshot

This part of the study displays the different interfaces on an E-Recruitment System as well as its definition that is useful for farther understanding.

Figure 4 shows the security box for the administrator. It provides username and password. To ensure security of the system, the administrator provides valid username and password to access the system. The applicant also has its own username and password. Figure 5 shows the main window of the system for administrator side that automatically displays the lists of applicants. At its left, list the menu of the system. At the upper right, it shows the

log-out button for the administrator. Figure 6 shows the profile of the second applicant that contains information such as full name, gender, address, contact number email address as well as the uploaded application letter. The upper right is the button for sending message if the administrator is interested to contact the applicant for the schedule for the interview. Figure 7 shows the dialog box for sending message. It provides the textbox for the recipient and the message area and submit button. This window is useful once the administrator is interested for the applicant and informing for the schedule for the initial interview. Figure 8 displays the jobs window where the administrator can add new job, view lists of vacant jobs, view applicants who applied for that job, the edit button for modification of the job's information and the send SMS button that use for sending messages to those who are registered applicants. Figure 9 shows the registration area for job. It is where the administrator can add new vacant job of the institution providing all the needed information such as position title, item number, salary grade, level, as well as the requirements for the job and its eligibility.

Figure 10 shows the dialog box for sending message. After posting new vacant job, the administrator can send messages by just clicking the button send SMS and this dialog box would pop up. This box is for selecting recipients based on the area of specialization. If the vacant job is intended for that particular department, the entire registered applicant in that department can receive message. Figure 11 shows the list of applicants on that specific job. The sequence of the applicants is based on highest educational attainment. If the administrator is interested for that particular applicant, the name has its link towards the applicant's information. Figure 12 shows the dialog box for editing job's information providing all the necessary information of the job. This window is needed once there are mistakes of inputs during the registration of the job. Figure 13 shows the main window for the user. This is the sample first interface that the user can view. It contains the institution image as well as its whereabouts, the mission, vision and the goals. Figure 14 shows the registration form for the applicant. It provides basic yet necessary information such as full name, gender, highest educational attainment, cell number address, then the option whether the applicant would apply as staff or faculty, email address, and password that would be needed in accessing the system. Figure 15 shows the window for all the available jobs offered by the institution. It has a capability for showing additional information and the "apply now" button once the viewer is interested for that job. Figure 16 shows the log-in box for the user. It provides e-mail address and password. The system can be accessed once the e-mail address as well as the password is valid.

Development and Testing

The system had gone series of testing and evaluation repeatedly. First testing was made by the students of Computer Science where there was someone who acted as an administrator and some users. The admin tried to post job and the users applied then, the admin tested the notification mechanism. The students who were there were amazed as to how the applicant received SMS from the administrator informing the schedule for the interview. Lastly, testing was made by some schoolmates from college where they are very impressed when they received SMS in their cellphone from the system. They also give suggestion such as uploading their personal made resume and application letter. They tried also to act as an administrator and give more comments and corrections. Alpha Testing was done in an in-house virtual user environment where it was done at the end of development. Minor design changes may be made as a result of such testing. Beta testing was typically done by end-users or others where anyone can obtain test and provide feedback on the application. The testing made as well as the positive response provides an assurance to the researcher that system can surely be effective and efficient in the target department.

Implementation Plan

The researcher is triggered to develop the system in the sense that it satisfies the needs of the user as well as the HR personnel. Testing and evaluations were made for the assurance of the system's functionalities and found out that it gives an exact response to the user based on what the users are expecting. During the implementation, Wide Area Network (LAN) facilities must be prepared, and XAMMP software must be installed. After the installation, user training must be followed for further knowledge.

CONCLUSION

This document presents the E-Recruitment System for Human Resource Management office of Surigao State College of Technology. Online Recruiting is more effective and has proven to be convenient for both HR Personnel and applicants. It is concluded that the system is effective in reaching potential employees for the system is an online so therefore anybody can see and visit the site and capable in sending message via SMS. The system also was efficient in tracking, filtering and facilitating selection process of applicants since it was able to sort applicants based on their highest educational attainment. Moreover, this study was reliable in managing applicant's information and job's records because of its centralized storage area. Distributing of data is fast and less expensive via SMS. Speedy and hassle-free in retrieving information is attainable. The system also provides security of the system because of its username and password and limitation of access.

RECOMMENDATIONS

After thorough study of an E-Recruitment System, the researcher recommended that the system should be used and be implemented to the Human Resource Management Office. To properly use it, hardware and software must be checked from time to time for data updating and modification. There must be a proper training for the personnel to avoid misuse of the system. In addition to enhancement, it should have an initial examination for the applicants to measure the level of intelligence and must able to have a face-to-face interaction for an online interview. Moreover, there must be a way wherein authenticity of the information posted or given by the applicants would be attained.

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The researcher would like to extend her profound gratitude to all those in one way or another, have made this study possible. To the Almighty Father, for His strength, wisdom, unending love and above all for inspiring her heart while making this study for without Him she is totally nothing; Dr. Merlyn M. Sanchez, the Chair of Graduate School who has been very supportive and understanding to researchers; Mrs. Teresita L. Toledo, who has been there extending her support and guidance to researchers, for the encouragement and knowledge as well as her patience correcting the document; Engr. Unife O. Cagas, who helped and shared her knowledge and ideas of this study; IT Faculty, who provided an support and shared their knowledge as to this study by providing suggestions and corrections; Family who provided endless support, love, prayer and encouragement; Lastly, to her husband Renz M. Buctuan who gave his full support, love, prayer and help for the success of her study.

REFERENCES

1. Barber, L., (2008). e-recruitment Developments;
2. Holm, A., (2010). *The Effect of E-recruitment On the Recruitment Process: Evidence from Case Studies of Three Danish MNCs;*
3. Masters, et al (2010). *E-Recruiting with SAP ERP HCM Bonn, (PP. 23-25);*
4. Mcqueen, R., (2011). *Recruitment Strategies Managing/Effecting The Recruitment Process;*
5. Parry, et al., (2008). *Implementation of e-recruitment;*
6. Pin, et al., (2011). *Internet Recruiting Power: Opportunities and Effectiveness;*
7. Plessis, A., (2012). *Effectiveness of e-recruiting: empirical evidence from the Rosebank business cluster in Auckland, New Zealand;*
8. Tjoeng, A., (2013). *Rethinking the Job Title in your Advertisement: Attracting Talent through Strategic Titles.*
9. DGF. *How to Make a Conceptual Framework for a Thesis.* Retrieved from www.ehow.com/how_8382973_make-conceptual-framework-thesis.html, October 27, 2013;

10. Hansen, P., *Incorporating SMS into E-Recruitment & Marketing – The Benefits.* Retrieved formwww.scouterecruit.com/incorporating-sms-into-e-recruitment-marketing-the-benefits, April 5, 3013;
11. Jobs DB Dimension., (2011).*E-Recruitment Strategy and Problems of Traditional Recruitment Process*;
12. Rahul, R., (2012). *Comparison between traditional and online recruitment.* Retrieved from www.projectguru.in/publications/a-comparison-between-traditional-and-online-recruitment-methods;
13. WIPO.,(2010). *E-Recruitment System;*
14. WorkplaceInfo., *Traditional recruitment methods — a failure: research.* Retrieved from www.workplaceinfo.com.au/recruitment/problems-and-challenges/traditional-recruitment-methods-a-failure-research, August 24, 2011.

Table : Gantt Chart Schedule of Activities

ID	Task Name	October	November	December	January	February	March
1	Identifying Establishment						
2	Conducting Interview						
3	Planning and understanding the problem						
4	Identifying Project Title						
5	Searching and downloading Related Literature						
6	Making document Chapters 1-2						
7	Developing the system						
8	Continuing document Chapters 3-5						
9	System Evaluation and Testing						
10	Preparing for Oral Defense						
11	Performing Oral Defense						
12	Revising document and system						
13	Submitting final document and system						

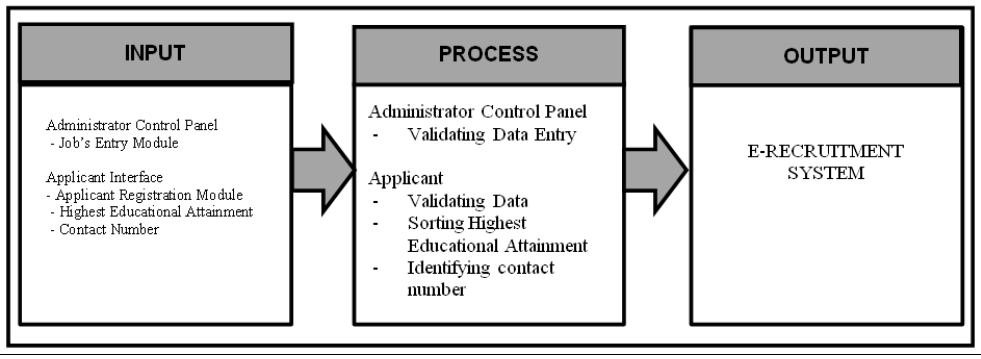

Figure 1: Flow of the Study

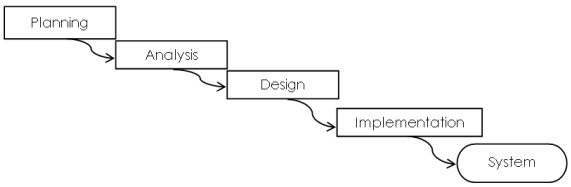

Figure 2: Waterfall Development

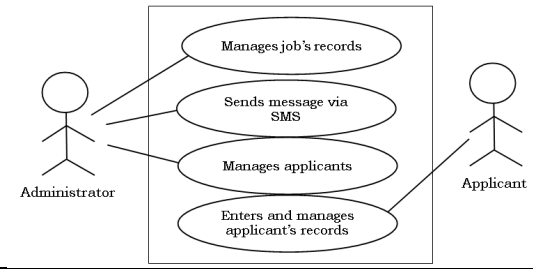

Figure 3: Use Case Diagram of E-Recruitment System

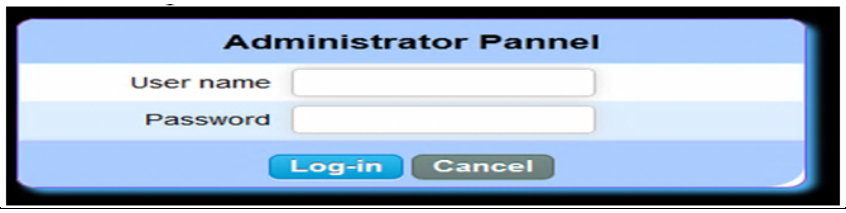

Figure 4: Administrator Pannel

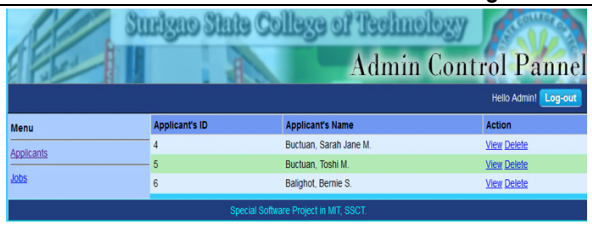

Figure 5: Main Window of E-Recruitment for Admin

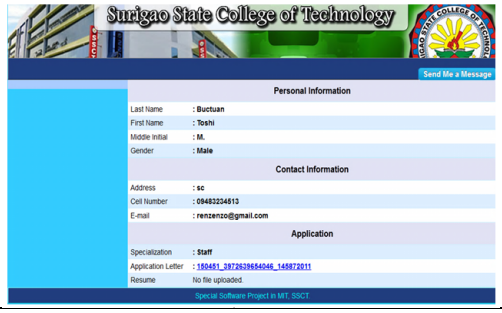

Figure 6: The Profile of the second applicant

Figure 7: Sending Message Dialog Box

Figure 8: Jobs window

Figure 9: Job Registration Dialog Box

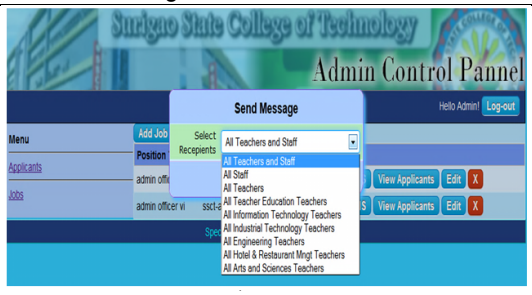

Figure 10: Dialog box for sending message

Figure 11: Dialog box for the list of applicants

Figure 12: Dialog Box for Editing Job's Information

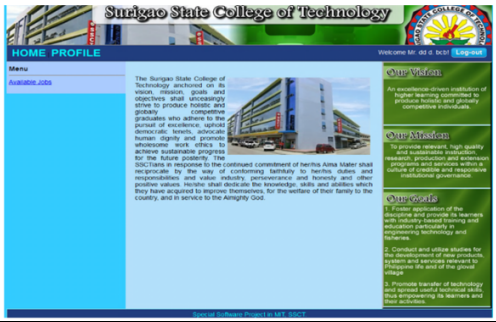

Figure 13: Main Window for the User

Figure 14: Registration Form

Figure 15: Window for available jobs

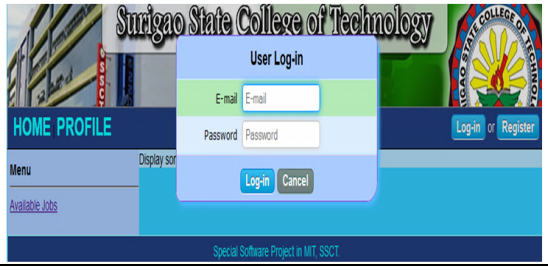

Figure 16: Log-in Box for user

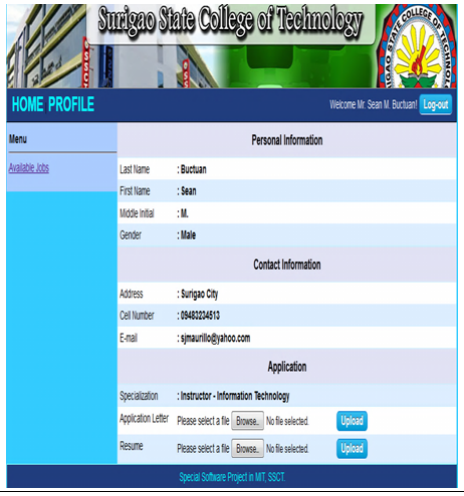

Figure 17: Applicant's Profile Window